Functional divergence between *Vachellia* and *Senegalia* could underpin differences in invasiveness and Eltonian niche partitioning in African savannas

By Joel Robin Lewis, June 2019

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In loving memory of a great man and mentor, my Oupa, Prof. P. J. Smit

Declaration:

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Table of Contents:

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Abstract

For several decades *Vachellia* and *Senegalia* have been assumed to be ecological equivalents in African savannas. Their supposedly close evolutionary relationship has, however, been totally revised in light of recent molecular phylogenetic data. These data highlight the deep divergence (± 30 Ma) between these genera and also their independent transitions into African savannas. Distant divergence and independent transitions into savannas between *Vachellia* and *Senegalia* suggest their possible ecological niche divergence, which could explain the observed differences in invasiveness between them. This thesis provides the first ecophylogenetic study to assess the level of ecological niche divergence between these economically, socioeconomically and ecologically important tree genera in Africa. I address this question by first determining the scale(s) of coexistence between *Vachellia* and *Senegalia* and determine their phylogenetic community structure at both the landscape-regional scale and the plot-scale. I then conduct a large glasshouse experiment using seven species from each genus to determine conserved functional differences between them during seedling establishment in the presence, and also the absence, of grass competition. While I find evidence of some Grinnellian niche differentiation between *Vachellia* and *Senegalia* at the landscape-regional scale, their greater-than-expected co-occurrence in plots suggests they generally partition an Eltonian niche in savannas. Glasshouse trait data also reveal that *Vachellia* has a conserved strategy for rapid vertical growth when free from grass competition and *Senegalia* has a conserved root tissue density response to grass competition. I argue that the rapid growth (bolting) strategy of *Vachellia* in the absence of grass competition provides a functional explanation for why this genus is observed to be relatively more invasive in grassy ecosystems. I also argue that the root tissue density response of *Senegalia* enables stable coexistence at the plot scale with *Vachellia* as it increases competitive ability among grasses leaving establishment less tied to times of low grass biomass. I conclude that this study provides overwhelming support for ecological niche divergence between *Vachellia* and *Senegalia* and that this divergence may underpin their observed differences in invasiveness. That the coexistence of these genera is largely attributable to Eltonian niche partitioning also provides fresh support for the contention that tree-tree competition structures tree communities in African savannas. Finally, niche divergence suggests that, when promoting diversity and managing invasion, *Vachellia* and *Senegalia* species will require different management practices.

Chapter 1:

General introduction

Savanna is an expansive tropical-to-subtropical vegetation type characterized by a continuous understorey C_4 grass layer and a discontinuous overstorey C_3 tree layer (Frost *et al.*, 1986; Scholes and Archer, 1997; Rutherford *et al.*, 2006). The diversity of savanna vegetation, therefore, owes much to the factors that enable the coexistence of grasses and trees, an understanding of which, is central to the preservation and management of this iconic biome (Sarmiento, 1984).

In southern African savannas mean annual precipitation (MAP) ranges between 300 mm and 1200 mm and is strongly seasonal (Frost *et al.*, 1986; Rutherford *et al.*, 2006), with a wet season from November to April and a dry season from March to October (Frost *et al.*, 1986; Rutherford *et al.*, 2006). Rainfall seasonality results in the shade-intolerant grasses reliably curing during the dry season, producing fuel loads that burn regularly (Frost *et al.*, 1986; Scholes and Archer, 1997; Rutherford *et al.*, 2006). High grass productivity during the wet season and consequent fires during the dry season tend to exclude fire-sensitive, shade-tolerant, tree species (Keeley and Rundel, 2005). This cycle of curing and burning expands and maintains savanna vegetation at the expense of more wooded, shady biomes (Keeley and Rundel, 2005; Charles-Dominique *et al*., 2015a). The occurrence of regular surface fires and the exclusion of deep shade has, consequently, allowed for the evolution of fire-tolerant and shade-intolerant tree species (Keeley and Rundel, 2005; Charles-Dominique *et al*., 2015a). Frost incidence in African savannas is also low, with average daytime temperatures around 32° C during the wet season and 20° C during the dry season (Rutherford *et al.*, 2006). As in savannas worldwide, nutrients are heterogeneously distributed across the landscape, generally leaching by erosion from ridgetops and accumulating in bottomlands (Rutherford *et al.*, 2006; Khomo *et al.*, 2011) and furthermore, soils are characteristically poor in nitrogen and phosphorus (Feral *et al.*, 2003; Venter *et al.*, 2003; Craine *et al.*, 2008).

Nitrogen and phosphorus limitation in soils present particular challenges for tree seedlings establishing within an almost-continuous, hyper-competitive grass layer (Cramer *et al*., 2007, 2010, 2012), especially in arid savannas (Ward *et al*., 2013). While direct resource-competition with grasses is capable of stalling the release of juvenile trees into adult size-classes (February *et al*., 2013a), seedling establishment is principally limited by root gaps in the grass layer (Wakeling *et al.*, 2014). In savannas, however, natural disturbance of the grass layer (Frost *et al.*, 1986; Scholes and

Archer, 1997), coupled with relatively higher nutrients and warmer temperatures compared to upland grasslands, ensures the presence of sufficiently-large gaps for tree seedling establishment to be successful (Wakeling *et al*., 2010, 2012). Both grasses and trees are primarily rooted in and utilise the shallow (< 20cm) soil horizons (February and Higgins, 2010; February *et al*., 2011, 2013b; Kulmatiski and Beard, 2013) which contain the vast majority of nitrogen (February and Higgins, 2010; February *et al*., 2011), a critical limiting-nutrient for tree seedlings (Cramer *et al*., 2010, 2012). Tree seedling establishment, therefore, initially requires investment in rapid taproot elongation (Brown and Archer, 1990; Bragg *et al*., 1993; Weltzin and McPherson, 1997; Wilson and Witkowski, 1998; Kambatuku *et al*., 2013; Lewis and February, unpublished) and/or symbiotic nodule formation (Cramer *et al.*, 2007) in addition to sufficiency of root gaps. Once successfully established as seedlings, however, savanna trees may persist for many decades trapped in small, non-reproductive (juvenile), size-classes, despite being regularly top-killed by fire and herbivory (Trollope, 1984; Bond and van Wilgen, 1996; Higgins *et al*., 2007; Sankaran *et al*., 2013; Staver and Bond, 2014). Even though the regular fires inherent to savannas keep juvenile trees from reaching reproductive size classes, these trees are extremely fire-tolerant, having evolved traits such as thick bark, epicormic buds and the use of stored starch reserves to aid in resprouting (Schutz *et al*., 2009; Staver *et al*., 2012; Charles-Dominique *et al*., 2015a; Charles-Dominique *et al*., 2015b). Savanna trees are also both chemically and physically defended against the many large mammalian herbivores typically found in this system (Cooper and Owen-Smith, 1986; Midgley *et al*., 2001; Hattas *et al*., 2011; Charles-Dominique *et al*., 2015c; Wigley *et al*., 2018, 2019). An almost constant threat of fire and large herbivores means that savanna trees must invest in rapid vertical growth, to allow juveniles to reach mature adult size classes beyond the reach of flames and herbivores (Wakeling *et al*., 2011; Sankaran, *et al*., 2013; Staver and Bond, 2014). In keeping juvenile trees from reaching maturity, regular fires and herbivores are thus important in maintaining the codominance of trees and grasses in the savanna (Sankaran *et al*., 2005; 2008).

The equilibrium between trees and grasses is, however, increasingly threatened by several factors, including improper management practices (Higgins *et al*., 1999; Hoffman and Todd, 2000; O'Connor *et al*., 2014), nitrogen deposition (Sankaran *et al*., 2008) and climate change (Wigley *et al*., 2010; Buitenwerf *et al*., 2012). These factors act together in complex ways to promote the phenomenon of bush encroachment (Ward, 2005; Wigley *et al*., 2009; O'Connor *et al*., 2014; Stevens *et al*., 2017). Bush encroachment, simply defined as the expansion of native woody species at the expense of grasses, poses one of the greatest threats to savanna biodiversity because it is taking place rapidly and is still poorly understood (Ward, 2005; O'Connor *et al*., 2014; Nackley *et al*., 2017). Although ecologists have identified which species are most invasive, debate still surrounds the primary cause

of bush encroachment and the functional traits which allow a single native species to become dominant (Ward, 2005; O'Connor *et al*., 2014). It is critical to address this research gap because bush encroachment can result in considerable economic losses in nature reserves via impairment of the visitor experience (Gray and Bond, 2013) and also in rangelands via land degradation (O'Connor and Crow, 1999; O'Connor *et al*., 2014). Currently, bush encroachment research is focused on factors that promote increases in tree density (Wigley, *et al*., 2010; Belay and Moe, 2012; Buitenwerf *et al*., 2012; O'Connor *et al*., 2014; Stevens *et al*., 2017) and the woody plant traits most likely to suppress grass productivity (Belay and Moe, 2015). In this research, however, the emphasis is often on adult trees, when one of the most important aspects of the invasion process is seedling establishment (Brown *et al.*, 1998). Successful seedling establishment is possible when seeds travel to and germinate in safe sites. These sites are gaps in the landscape with the appropriate environment to support the recruitment of (usually) a single adult individual (Falster *et al*., 2008), determined by a species' ecological niche (Soberón, 2007).

The ecological niche is a challenging concept that has been subject to much confusion over the years (Whittaker *et al*., 1973; Silvertown, 2004; Soberón, 2007). At the most basic level, a new niche is occupied when a species adapts to a novel environment through functional trait changes that become fixed over evolutionary time. These adaptations will include functional responses to differences in abiotic and biotic filters and/or their interactions (Whittaker *et al*., 1973; Soberón, 2007). When a species adapts to occupy a niche in a new habitat (landscape-regional scale) a suite of functional traits is usually necessary to deal with both the abiotic and biotic differences, whereas, when occupying a niche within the same community (plot scale) species mostly only require differences in traits relating to biotic filters (Whittaker *et al*., 1973; Soberón, 2007). These differences in niche are divided into a Grinnellian niche, which refers to the habitat in which a species lives and an Eltonian niche which refers to the resource-use of that species. These two niche concepts together, refer to the ecological niche of a species (Soberón, 2007).

The contrasting spatial scale of factors influencing a species Grinnellian or Eltonian niche means that ecological niche partitioning may be seen at both the plot scale and/or landscape-regional scale (Webb *et al.*, 2002; Cavender-Bares *et al.*, 2009). For niche partitioning to be stable, however, species must be able to avoid direct resource competition at the plot scale, as this competition is expected to drive inferior species to extinction in communities (MacArthur and Levins, 1967; Chesson, 2000). Where coexistence at the plot scale (Eltonian niche partitioning) is thus normally contingent on traits enabling avoidance of direct resource competition between species (Whittaker *et al*., 1973; Soberón, 2007). Coexistence at the landscape-regional scale (Grinnellian niche

partitioning) is contingent on the suite of traits which determine habitat differences, thus enabling indirect avoidance of competition between species (Whittaker *et al*., 1973; Soberón, 2007). A consideration of the scale at which species coexist is, therefore, critical for studies seeking to uncover the functional traits underpinning niche partitioning between species. Differences in traits enabling niche partitioning must, however, also be conserved with respect to phylogeny in order to support continued coexistence through evolutionary time (niche divergence) between species (Losos, 2008). Phylogeny, therefore, is an important further consideration when determining traits underpinning niche partitioning between species (Webb, 2000; Silvertown *et al*., 2001; Webb *et al*., 2002; Cavender-Bares *et al*., 2004, 2009; Slingsby and Verboom, 2006; Losos, 2008).

On account of their recent shared ancestry, closely related species are expected to possess similar traits and to occupy similar niches, a pattern termed *phylogenetic niche conservatism* (Prinzing *et al.*, 2001; Webb *et al.*, 2002; Donoghue, 2008; Losos, 2008; Cavender-Bares *et al.*, 2009; Crisp and Cook, 2012; Petitpierre *et al.*, 2012). In this context, closely-related species are expected to compete more intensely for resources, potentially limiting their ability to coexist stably at the plot scale (Silvertown *et al*., 2001; Cavender-Bares *et al*., 2004; Slingsby and Verboom, 2006). In these situations, plot-scale communities are expected to show phylogenetic overdispersion, a pattern in which coexisting species are more distantly related than expected by chance (Webb *et al.*, 2002; Cavender-Bares *et al.*, 2004). At the landscape-regional scale, however, niche conservatism means that closely related species tend to occur in similar habitats, producing a pattern of phylogenetic clustering (Webb *et al.*, 2002; Cavender-Bares *et al.*, 2004; Cardillo, 2011; Eiserhardt *et al.*, 2015). Niche conservatism may thus explain why members of some lineages are inherently more invasive than those of other lineages (O'Connor *et al*., 2014). Of the eight most invasive species identified in southern Africa, four are species of the genus *Vachellia* and only one is a species of the genus *Senegalia* (O'Connor *et al*., 2014). Additionally, across all 18 sites investigated by O'Connor *et al.* (2014), 90% of sites showed some encroachment by species of *Vachellia* while only 29% showed signs of encroachment by species of *Senegalia*. The study of O'Connor *et al.* (2014) suggests that a key ecological driver promoting bush encroachment is the creation of safe sites for *Vachellia* species relative to those of *Senegalia*.

Differences in the ecological behaviour of these lineages might be a direct consequence of their independent evolutionary trajectories since their divergence from a common ancestor, ca. 30 Ma (Bouchenak-Khelladi *et al*., 2010; Kyalangalilwa *et al*., 2013). Paleobotanical evidence shows that from the mid Eocene (± 46 Ma) to the late Oligocene (± 27 Ma), global cooling and increasing aridity resulted in forest contraction and fragmentation, which spurred the evolution of many open-

adapted plant taxa (Raven and Axelrod, 1974). One of these taxa, a deciduous mimosoid liana, is hypothesised to have been the most recent common ancestor of *Vachellia* and *Senegalia* (Ross, 1981). Molecular phylogenetic evidence suggests that this ancestral species occupied closed habitats (tropical rainforest, forest margin and woodland) in Africa or America (Bouchenak-Khelladi *et al.*, 2010). Having diverged ca. 30 Ma, *Vachellia* and *Senegalia* initially continued to evolve independently in closed habitats, entering open habitats (grasslands, savannas, savanna woodlands and deserts) only 16 Ma and 10 Ma, respectively (Bouchenak-Khelladi *et al.*, 2010). Distant divergence and independent transitions into African savannas between these genera highlights their independent evolutionary trajectories and possible niche divergence.

In this thesis, I test the hypothesis that *Vachellia* and *Senegalia* have evolved contrasting ecological niches/strategies since their divergence 30 Ma., and that this underpins their differential invasiveness in southern African savannas. To test this hypothesis, I first determine the scale at which they coexist in southern Africa by determining co-occurrence and phylogenetic community structure at both the landscape-regional scale and plot scale. I then conduct a large glasshouse experiment to quantify functional trait divergence between these lineages during seedling establishment in both the presence and absence of grasses.

Chapter 2:

Vachellia and *Senegalia* show Grinnellian niche overlap and Eltonian niche partitioning in southern African savannas

Introduction

Ecosystems comprise a diversity of species-rich communities which occasionally assemble non-randomly through a variety of scale-dependent ecological drivers (Whittaker *et al*., 2001; Willis and Whittaker, 2002). Species richness at the landscape or regional scale (gamma richness) is a product of species richness within plots (alpha richness) and species turnover between plots (beta richness) (Whittaker *et al*., 2001; Jost, 2013). Thus, where alpha richness is contingent on species coexistence at the plot scale, gamma richness is contingent on species coexistence at both the plot and landscape-regional scales (Whittaker *et al*., 2001). While coexistence at the landscape-regional scale is generally facilitated by the evolution of trait differences which differentiate the Grinnellian niches of species, coexistence at the plot scale is enabled by traits which underpin Eltonian niche differentiation (Whittaker *et al*., 1973; Soberón, 2007). Knowledge of the spatial scale at which species coexist is, therefore, central to understanding the process underpinning species coexistence and diversity in a landscape. This, in turn, is important because diversity has been forwarded as the primary driver of ecosystem stability (McCann, 2000; Hooper *et al.*, 2005).

Since niche-related traits often show a phylogenetic signal, closely related species tend to have similar functional attributes and resource requirements (Webb *et al.*, 2002; Losos, 2008; Cavender-Bares *et al.*, 2009; Crisp and Cook, 2012; Petitpierre *et al.*, 2012; Cornwell *et al.*, 2014). As such, competition for resources may often be strongest between closely related species, which sometimes limits the ability of close relatives to coexist stably at the plot scale (Webb, 2000; Cavender-Bares *et al.*, 2004, 2009; Slingsby and Verboom, 2006). At the landscape-regional scale, however, coexistence of closely related species is facilitated by habitat heterogeneity and dispersal limitation (Prinzing *et al.*, 2001; Webb *et al.*, 2002; Cavender-Bares *et al.*, 2009; Cardillo, 2011). Determining the phylogenetic structure of plot-scale communities and landscape/regional-scale species assemblages can, therefore, provide valuable insights into the processes structuring species coexistence and diversity at these scales (Webb *et al.*, 2002; Cavender-Bares *et al.*, 2009; Mouquet *et al.*, 2012). For example, a pattern of phylogenetic overdispersion in a plot suggests an strong competition for resources among closely related species resulting in the exclusion of the inferior (closely-related)

competitors and coexistence of distantly related species. As closely related species are too similar in resource use to coexist, such a community is likely enabled by differences in resource use or differences in Eltonian niche between distantly related species. A pattern of phylogenetic clustering however, suggests a high degree of habitat filtering relative to competition for resources and thus, closely related species (sharing the essential traits for survival) can coexist. Coexistence between distantly related species, therefore, only happens at a greater scale, as conserved Grinnellian niche differences mean they occur in different habitats.

To date, there has been little research assessing niche divergence and coexistence of savanna tree lineages in Africa. In principle, the high gamma richness of *Vachellia-Senegalia* (native African *Acacia s.l.* species; Ross, 1979) in African savannas may be a product of Grinnellian niche differences between *Vachellia* and *Senegalia*, allowing species of these two genera to share different habitats across the region or landscape (Scenario 1). Alternatively, high gamma diversity of *Vachellia-Senegalia* may be a consequence of Eltonian niche partitioning between these genera, allowing species to coexist at the plot-scale and diversify in similar regions (Scenario 2). Scenario 1 and 2, have opposite predictions for the relatedness of *Vachellia-Senegalia* species coexistence within landscape-regional assemblages and plot communities. Where scenario 1 predicts phylogenetic clustering at the landscape-regional scale and/or within plot-scale communities, scenario 2 predicts phylogenetic overdispersion within plot-scale communities. In this chapter, I assess these alternatives by determining the phylogenetic structure of *Vachellia-Senegalia* assemblages at both the regional (species present within quarter degree grid cells within southern Africa) and plot (species present within 20 X 20 m plots within the Kruger National Park) scales. I also use these datasets to determine Grinnellian niche overlap between *Vachellia* and *Senegalia* at a regional and landscape scale, respectively, using publically available climate and soil GIS layers.

Methods

Phylogenetic inference

Phylogenetic relationships and associated divergence times of *Vachellia-Senegalia* species were estimated in BEAST version 1.4.6 (Drummond and Rambaut, 2007) using the alignment of Bouchenak-Khelladi *et al.* (2010) but augmented with GenBank-sourced sequences of *Vachellia haematoxylon* Willd. (GenBank accessions AF523189, EU4400241, JQ230192), *V. natalitia* E.Mey. (JX517566, JQ230233, JQ278603) and *Senegalia hereroensis* Engl. (JQ230132, JQ265939, JQ230184) which were aligned using BioEdit version 7.0.5 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). The final alignment contained sequences representing the *matK*, *trnL-F* and *trnH-psbA* regions of the chloroplast genome. Calibration priors were the same as those used by Bouchenak-Khelladi *et al.*

(2010), each calibration being specified as a lognormal prior whose zero-offset, log(mean) and log(standard deviation, sd) were set such that prior had a median corresponding to the most probable age of the relevant reference fossil. Following Bouchenak-Khelladi *et al.* (2010), calibrations were applied to the root node of the phylogeny (zero-offset = 54.0 Myr; log(mean) = 1.792 Myr; log(sd) = 1.0 Myr), the crown node of *Mimosoideae* (zero-offset = 50.0 Myr, log(mean) = 1.610 Myr; log(sd) = 1.0 Myr), and the crown node of *Senegalia* (zero-offset = 13.5 Myr, log(mean) = 0.405 Myr; log(sd) = 1.0 Myr). Two independent Markov chain Monte Carlo (MCMC) chains of 5 x 10^7 generations were run, sampling every 1000^{th} generation. TRACER version 1.6 (Drummond and Rambaut, 2007) was used to check for run convergence to determine the length of burn-in. The first 5,000 samples obtained from each run were then discarded as burn-in, yielding a total of 90,000 samples which were summarized as a maximum clade credibility tree using TreeAnnotator (Drummond and Rambaut, 2007). The phylogeny was then trimmed to contain only those species used in subsequent analyses at a regional and then a local scale. Ancestral transitions from closed-type (i.e. tropical rainforests, forest margins and woodlands) to open-type (i.e. grasslands, savannas, savanna woodlands and deserts) habitats were then inferred from Bouchenak-Khelladi *et al.* (2010) for the reduced trees. Presented trees were created using FigTree version 1.4.2 (Rambaut, 2006).

Source and preparation of QDS and plot distribution data

For Quarter Degree Square (QDS) distribution data, all records of native *Acacia s.l.* species for South Africa, Namibia, Botswana and Swaziland were accessed online from the PRECIS database (http://posa.sanbi.org/intro_precis.php), courtesy of the South African National Biodiversity Institute (SANBI), before being assigned to *Vachellia* or *Senegalia* on the basis of phylogeny (Kyalangalilwa *et al*. 2013). All records from botanical gardens were excluded, as were records collected at a spatial scale greater than a quarter degree grid square cell (QDS). This resolution amounts to an area of approximately 2500 km^2; thus, QDS inevitably include different habitats occurring under relatively similar climatic conditions. This dataset is, therefore, adequate to determine whether regional climatic preferences differences exist between species of these genera across southern Africa, with an arid climate in the west and a more mesic climate in the east (Rutherford *et al.*, 2006). Also, except for *S. montis-usti*, whose six records adequately captured its small range on the Brandberg Mountains of Namibia, all species with fewer than ten records were excluded. The resulting dataset contains 13 species of *Senegalia* and 20 species of *Vachellia* (Table 1, Supp. 1), with data for a total of 934 QDS. While not strictly savanna species (Palgrave and Palgrave, 2002), *S. kraussiana* (a strong creeper occurring in scrub and forest) and *S. schweinfurthii* (a robust scrambler occurring in riverine forest and woodland) were included to capture the entire ecological range of *Senegalia*.

For plot distribution data, a dataset of woody species in 1985 homogenous 20 m x 20 m plots across the Kruger National Park (KNP; Venter, 1990) was reanalysed by first removing all non-*Acacia s.l.* (including *Faidherbia albida*) and duplicate entries and then reclassifying the remaining species as *Vachellia* or *Senegalia* as done for the QDS dataset. This final dataset includes eight species of *Vachellia* and six species of *Senegalia* (Table 1, Supp. 1) recorded from a total of 834 plots. *Senegalia schweinfurthii* was not removed from this dataset for the same reason as highlighted above. *S. mellifera* and *V. luederitzii*, however, were removed given that these species were only represented by only one or two entries, respectively. This dataset represents a local community dataset, given that plots were homogenous and confined to particular habitats, providing an adequate test for Eltonian niche partitioning between species. The sampling scale (20 m x 20 m) is also well within the neighbourhood scale (<100 m) defined by Webb *et al*. (2002); thus, the composition of these communities is likely to be determined by interactions between individuals and the process of neighbour (competitive) exclusion.

Determination of Grinnellian niche overlap between Vachellia and Senegalia

To determine Grinnellian niche overlap between *Vachellia* and *Senegalia* at a regional scale, QDS distribution data were used to characterize the climatic niches of their species in a GIS context. Since precipitation and temperature variables are both major determinants of savanna tree species distribution at the regional scale (Burke, 2006; Wakeling *et al*., 2012; Stevens *et al*., 2014), I focussed on the following variables: minimum temperature of the coldest month, maximum temperature of the warmest month, annual temperature range, precipitation during the wettest quarter, precipitation during the driest quarter and precipitation seasonality. These data were accessed online from the WorldClim (version 2) database (http://worldclim.org/version2). Although the data were downloaded at a 5 arc-minute resolution, they were aggregated by a factor of 3 (15 x 15 arc-minutes) using the *raster* package version 2.5-8 (Hijmans, 2016) in R in order to match the QDS resolution. The value of each aggregated cell was calculated as the mean of cells included from the raw raster layer. For each species, the bioclimatic data corresponding to each QDS in which it occurred were then extracted using the *raster* package. Where NA values were extracted (e.g. when points fell on the ocean), descriptions of locality data were used to determine more accurate QDS coordinates using Google Earth version 7.3.1 (Google LLC, California, United States).

To determine landscape-scale Grinnellian niche overlap between *Vachellia* and *Senegalia,* plot distribution data were used to characterize climatic/habitat niche differences across KNP. For

analyses at this scale, WorldClim data were downloaded at a 30 arc-second resolution. Annual temperature range and precipitation seasonality were, however, dropped because of low variance across the park and replaced by elevation above sea level and fire frequency, due to the important role that these variables play in determining tree species' distribution at the landscape scale (Bond *et al*., 2001; Venter *et al*., 2003; Colgan and Asner, 2017). KNP fire data were accessed online from ORNL DAAC as .shp files of every fire that burnt between 1992 and 2001 (Woods and Govender, 2004). This GIS dataset was chosen on account of being closest (temporally) to the data collection of Venter (1990). These fire data were then rasterised into one layer of counts and following this, the total number fires burning during the 1992 to 2001 period were extracted for each plot using the *raster* package in R. Extracted data were then turned into frequency data by dividing the total number of fires by 9 (years). Elevation data are 1 km resampled Shuttle Radar Topography Mission (SRTM) data downloaded (http://www.cgiar-csi.org/data/srtm-90m-digital-elevation-database-v4-1#download) from the Consortium for Spatial Information. Differences in the geological/pedological association of genera and their species co-occurrence were also determined at this scale using a dataset for geology and soils within the KNP (Venter, 1990). Soils for this analysis were broadly categorised into basalt, granite or shale, based on the parent bedrock. To test whether *Vachellia* and *Senegalia* and/or their species co-occurrence showed preference for a particular soil type I used chi-squared goodness of fit tests. For these tests, expected values were calculated as the proportion of all plots sampled on that soil type multiplied by the total number of sites in which each genus (or co-occurrence) was found.

To test for differences in Grinnellian niche variables between *Vachellia* and *Senegalia*, I used a generalized linear mixed effects model (GLMM) treating genus as a fixed effect and species as a random effect nested within genus. GLMM's were run using the *lme4* package version 1.1-15 (Bates *et al*., 2015) in R. Significant differences were assessed using p-values generated using Satterthwaite's degrees of freedom method as implemented in the *lmerTest* package(Kuznetsova *et al*., 2017). To account for phylogenetic non-independence between species, I also fitted Ornstein Uhlenbeck (OU) models of character evolution to assess whether or not the two lineages have the same selective optima for a given trait (Hansen, 1997; Butler and King, 2004). OU models were run using species means, with significant differences between *Vachellia* and *Senegalia* being determined by comparing the Akaike information criterion (AIC) scores of a single optimum model (OU1) and a two-optimum model (OU2, fitted to *Vachellia* and *Senegalia*). The best model was then selected based on its AIC score. If OU2 had an AIC score <2 compared to OU1, it was considered as strong evidence of different selection optima; i.e. trait divergence and conservatism between *Vachellia* and

Senegalia (Burnham and Anderson, 2002). OU models were fitted with trimmed phylogenetic trees for both the QDS and plot data using the *hansen()* function from the *ouch* package version 2.11-1 (King and Butler, 2009). For both GLMM and OU analyses of QDS data raw data were used for all climatic variables, except for precipitation during the drest quarter, which was log-transformed to ensure normality of residuals. For these same analyses of plot data only raw data were used, as the residuals of each variable were roughly normally distributed.

Finally, using both the QDS- and plot-scale datasets, Grinnellian niche differences between genera were visually assessed by plotting species richness onto a Mean Annual Precipitation (MAP) raster layer using R, treated identically to the other climatic variables analysed at each respective scale, to determine if their diversity was restricted to different geographical areas. Correlations of species richness between *Vachellia* and *Senegalia* were then plotted to determine the shape of this relationship at both these scales. QDS/plots containing no species of either genus were included in these comparisons using back-of-the-envelope calculations. For the QDS-scale dataset the total number of QDS in South Africa, Namibia, Botswana, Zimbabwe and Swaziland containing no records of either *Vachellia* or *Senegalia* was determined to be 2940 - 934 = 2006, while for plot-scale dataset the number was determined to be 1985 - 834 = 1151.

Analyses of phylogenetic community structure

Phylogenetic patterns of species coexistence were assessed for both the QDS and plot datasets. To do this I first produced, for each dataset, a matrix recording the presence/absence of all *Vachellia* and *Senegalia* species in each QDS or plot. Pairwise phylogenetic distances between all species were then summarized as a phylogenetic distance matrix using the *cophenetic.phylo()* function in the *ape* package version 3.5 (Paradis *et al*., 2004) in R version 3.3.1 (R Core Team, 2016). The relationship between species co-occurrence and phylogenetic distance was then quantified using the $R_{PD\text{-}DO}$ metric (Slingsby and Verboom 2006) which accounts for difference in the prevalence of species. This measure of pairwise co-occurrence was then plotted against corresponding pairwise phylogenetic distances to assess whether phylogenetic relatedness significantly influenced co-occurrence. Following Cavender-Bares *et al.* (2004), linear regression was used to determine the strength of correlation between these two variables. Since pairwise-distance matrix comparisons of this type render the individual data points non-independent, the significance of the correlation coefficient was assessed against a null distribution of coefficients. Null distributions were generated by reshuffling species identity in all QDS/plots while maintaining community richness constant 999 times and recalculating the coefficient each time.

In a second analysis, the phylogenetic structure of communities of locally co-occurring species was assessed using the standard effect size of mean phylogenetic distance (SES-MPD). To do this, the SES-MPD of each QDS/plot was calculated as the observed mean phylogenetic distance (MPD) minus the mean MPD of 999 randomised communities. Randomised communities were created using the independent-swap algorithm, maintaining species occurrence frequency and sample species richness, using the *picante* package version 3.5 (Kembel *et al*., 2010) in R version 3.3.1 (R Core Team, 2016). The difference between observed MPD and null MPD was then divided by the standard deviation in MPD across the randomised communities. The SES-MPD scores of all plots were then plotted as a histogram, and the mean SES-MPD then compared against a null value of zero using a non-parametric Wilcoxon Ranked Sum Test (data non-normally distributed).

In a third analysis, I determined whether the co-occurrence of *Vachellia* and *Senegalia* species was greater than expected by chance. To do this, I determined the standard effect size of Pielou's Evenness (SES-E) in all QDS or plots having two or more species. For this purpose, the determination Pielou's Evenness (E) was based on the numbers of species of each genus occurring within a given QDS or plot. Evenness was calculated using the formula $E = \frac{H'}{H'_{max}}$ where H' is the Shannon-Wiener diversity index calculated as $H' = -\sum_{p_i}^{R} p_i \ln(p_i)$, with p_i being the proportion of species belonging to the i^{th} genus, and H'_{max}, when both genera are present equally, is calculated as;

$$H'_{max} = -[\tfrac{1}{2}\ln\left(\tfrac{1}{2}\right) + \tfrac{1}{2}\ln\left(\tfrac{1}{2}\right)]$$

and when genera were not present equally;

$$H'_{max} = -[\frac{(n_{species}/2)+0.5}{n_{species}}\ln\left(\frac{(n_{species}/2)+0.5}{n_{species}}\right) + \frac{(n_{species}/2)-0.5}{n_{species}}\ln\left(\frac{(n_{species}/2)-0.5}{n_{species}}\right)].$$

For example, a plot with one species of *Senegalia* and two *Vachellia* has an evenness of;

$$E = \frac{H'}{H'_{max}} = \frac{-[\frac{1}{3}\ln\left(\frac{1}{3}\right)+\frac{2}{3}\ln\left(\frac{2}{3}\right)]}{-[\frac{(3/2)+0.5}{3}\ln\left(\frac{(3/2)+0.5}{3}\right)+\frac{(3/2)+0.5}{3}\ln\left(\frac{(3/2)+0.5}{3}\right)]} = 1.$$

Determination of SES-E was done in the same manner as for mean phylogenetic distance, with the SES-E scores of all plots again then being plotted as a histogram and their mean assessed against a null of zero (phylogenetic randomness). SES-E was later plotted in a on a MAP raster layer using R to determine if clustered and/or overdispersed QDS/plots showed any geographical pattern.

Results

Phylogenetic associations of Vachellia and Senegalia

The phylogenetic tree presented agrees with that of Bouchenak-Khelladi *et al.* (2010) in corroborating the deep evolutionary divergence (29.44 [22.42, 36.26] Ma; median [95% HPD

interval]) between *Vachellia* and *Senegalia*, and demonstrating the independent and asynchronous transitions of these genera into open, savanna vegetation in Africa. Where open-adapted *Vachellia* species appeared as early as 13.87 [9.09, 19.95] Ma, open-adapted *Senegalia* species appeared 9.17 [5.82, 13.28] Ma (Fig. 1).

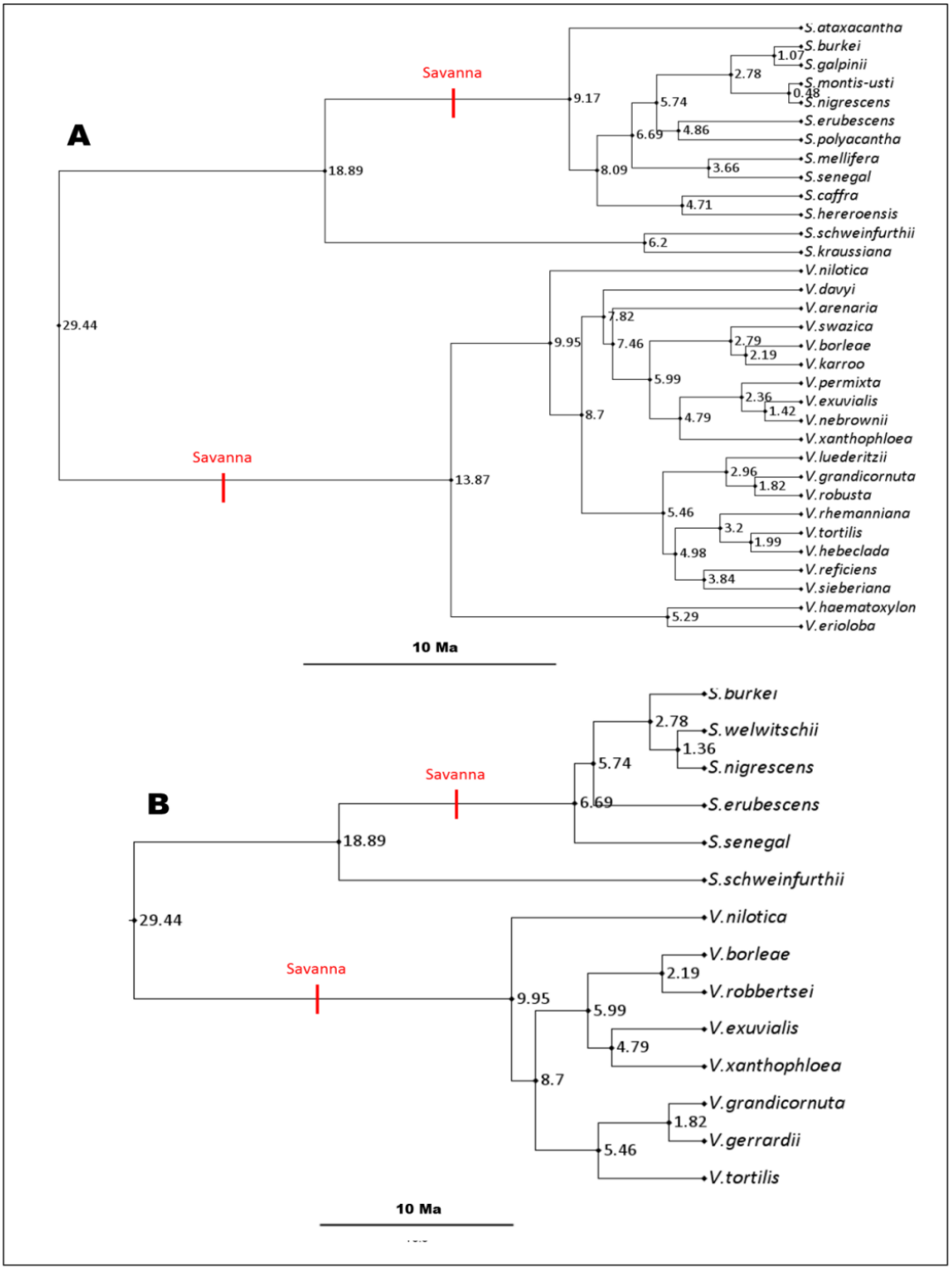

Figure 1: Phylogenetic trees for 20 species of *Vachellia* and 13 species of *Senegalia* used in the regional-scale analysis (**A**) and for the eight species of *Vachellia* and six species of *Senegalia* used in the plot-scale analysis (**B**). Node ages (Ma) are annotated, as are transitions from closed to open (≈ savanna) habitats following a larger phylogenetic analysis conducted by Bouchenak-Khelladi *et al.* (2010).

Grinnellian niche overlap and phylogenetic community structure between Vachellia and Senegalia

Across southern Africa, QDS data reveal that *Vachellia* and *Senegalia* are largely overlapping in their distributions, with a primary centre of diversity in the bush veld (North West Province) and low veld (Limpopo Province) regions of South Africa and secondary centres around Windhoek in Namibia and in central Botswana (Fig. 2). *Vachellia*, however, contains a further centre of diversity in the Kalahari region of South Africa and southern Botswana, and also in the Caprivi Strip in northern Namibia (Fig 2A). In general, *Vachellia* appears to enjoy greater species richness in QDS and a slightly wider range across southern Africa compared to *Senegalia* (Fig 2). Comparison of species richness of *Vachellia* and *Senegalia* in QDS reveals a strong positive relationship and shows that for QDS containing more than one species, mixed species assemblages (species from both genera) are far more common than unmixed assemblages with the same richness (Figure 2C). Despite differences in distribution between genera, however, neither GLMM nor OU analyses reveal any difference in the climatic environments occupied by these genera at the regional scale (Table 1). This high overlap in Grinnellian niche is likely attributable to the high proportion of QDS (344 QDS = 36.8%) found to contain species from both genera.

Closer inspection of the overall phylogenetic structure of QDS assemblages reveals a general trend towards overdispersion, with mean SES-MPD (mean = 0.16, median = 0.41) and mean SES-E (mean = 0.17, median = 0.51) being significantly positive ($P < 0.001$; Fig. 3C & D). Although a plot relating $R_{PD\text{-}DO}$ pairwise species co-occurrence index to phylogenetic relatedness appears triangular with a positively sloping hypotenuse, linear regression identified the correlation as non-significantly different from the null expectation (rank [obs R^2] = 659, P = 0.34; Fig. 3B). In contrast to the general pattern of phylogenetic overdispersion, some QDS with (uncharacteristically) strong clustering were observed (large negative values in Fig. 3C & D). While SES-E analysis identified no individual QDS as deviating significantly ($P > 0.05$) from null expectation, SES-MPD analysis revealed two significantly clustered and one significantly overdispersed QDS. Strongly clustered QDS were always dominated by species of *Vachellia* and found to be localised to the escarpment and Highveld regions of Limpopo Province, South Africa and also to the Kalahari region of South Africa and southern Botswana (Fig 3A), where *Senegalia* was absent or showed only low richness (Fig 2B). Plots showing overdispersion (evenness) were found to be highly concentrated in the KwaZulu-Natal Province, the bushveld region of the North West Province and the low veld region of Limpopo Province, South Africa (Fig 3A).

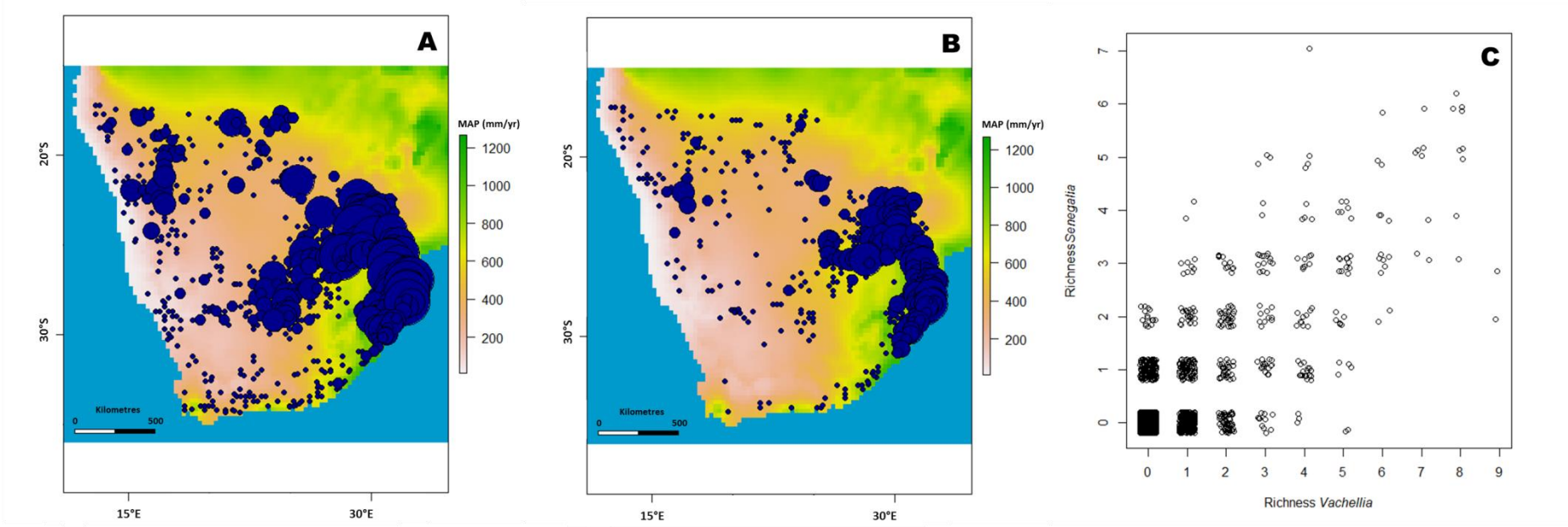

Figure 2: Distribution and richness of *Vachellia* (**A**) and *Senegalia* (**B**) across southern Africa and the correlation of their richness in QDS (**C**). Sizes of points are proportional to the number of species found in a QDS (**A** & **B**). Background layer shows Mean Annual Precipitation (MAP) at QDS resolution and light blue tiles are ocean (**A** & **B**).

Table 1: Raw mean (of species means) ± SE between *Vachellia* and *Senegalia* for environmental variables determined at both the regional and landscape scale in southern Africa and the Kruger National Park, respectively. P-values represent the result of GLMM analysis and ΔAIC is the difference between OU1 and OU2 AIC scores. Positive ΔAIC >2 suggests divergent selection and conservatism between these lineages.

	Vachellia	***Senegalia***	**P-val**	**ΔAIC**
Regional Scale				
Maximum Temperature of the Warmest Month (°C)	32 ± 0.1	32 ± 0.1	0.56	-1.6
Minimum Temperature of the Warmest Month (°C)	5.9 ± 0.1	6.6 ± 0.1	0.48	-1.6
Annual Temperature Range (°C)	26 ± 0.1	25 ± 0.1	0.41	-1.3
Precipitation during the Wettest Quarter (mm)	302 ± 3	321 ± 3	0.97	-2.0
Precipitation during the Driest Quarter (mm)	33 ± 1	35 ± 1	0.80	-2.0
Precipitation Seasonality (CV)	76 ± 1	77 ± 1	0.92	-2.0
Landscape Scale				
Maximum Temperature of the Warmest Month (°C)	32 ± 0.1	32 ± 0.1	0.68	-2.00
Minimum Temperature of the Warmest Month (°C)	8 ± 0.1	8 ± 0.1	0.78	-2.00
Precipitation during the Wettest Quarter (mm)	308 ± 1	303 ± 1	0.90	-1.90
Precipitation during the Driest Quarter (mm)	29 ± 0.3	28 ± 0.3	0.23	0.58
Elevation (m)	314 ± 3	307 ± 3	0.40	-1.49
Average Fire Frequency (fires/year)	0.17 ± 0.005	0.16 ± 0.005	0.72	-2.00

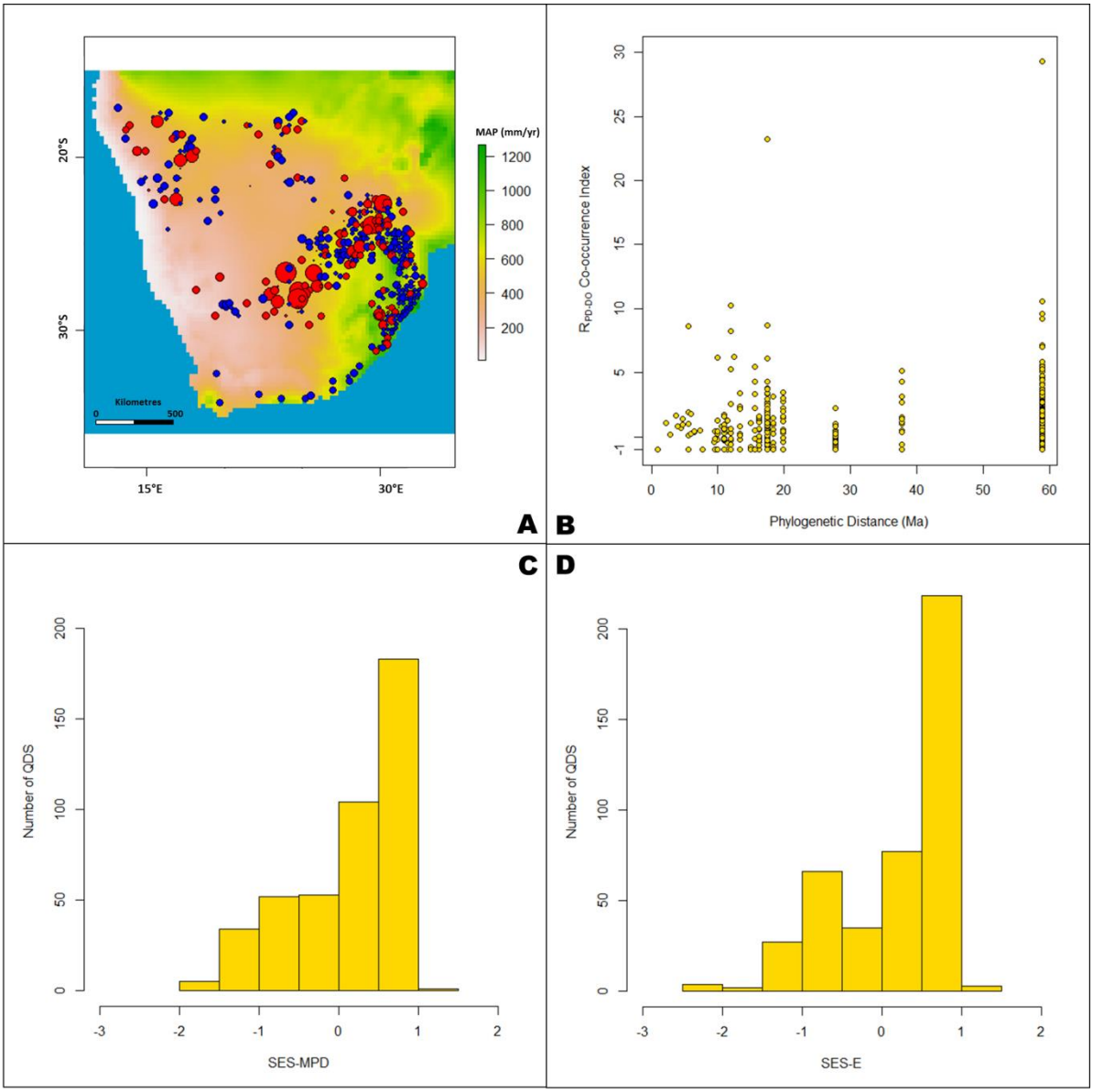

Figure 3: Regional-scale phylogenetic structure of *Vachellia-Senegalia* assemblages in southern Africa. Distribution of QDS showing evenness (blue) or clustering (red) where the size of points is proportional to the absolute value of SES-E (**A**). Background layer shows Mean Annual Precipitation (MAP) at QDS resolution and light blue tiles are ocean (**A**). A plot showing a non-signficant, broadly triangular relationship between R_{PD-DO} co-occurrence index and phylogenetic distance of all species pairs (**B**). Histograms showing significant and positive observed SES-MPD (**C**) and SES-E (**D**) across all sampled QDS.

Across the KNP, plot data identify *Vachellia* and *Senegalia* as having largely overlapping distributions, with the majority of their species richness being in the south and centre of the Park (Fig. 4A & B). *Vachellia*, however, has the highest species richness localised in the south, below 25°S compared to *Senegalia* which attains its highest species richness in the centre around 24° 25'S (Fig 4A & B). *Senegalia* also appears to have a greater distribution into the *Colophospermum mopane* dominated areas stretching north of the Olifants River to the Limpopo River, especially on the granite soils in the west (Fig 4B). Comparison of species richness of *Vachellia* and *Senegalia* in plots again reveals a positive relationship, with mixed species plots being far more common compared to

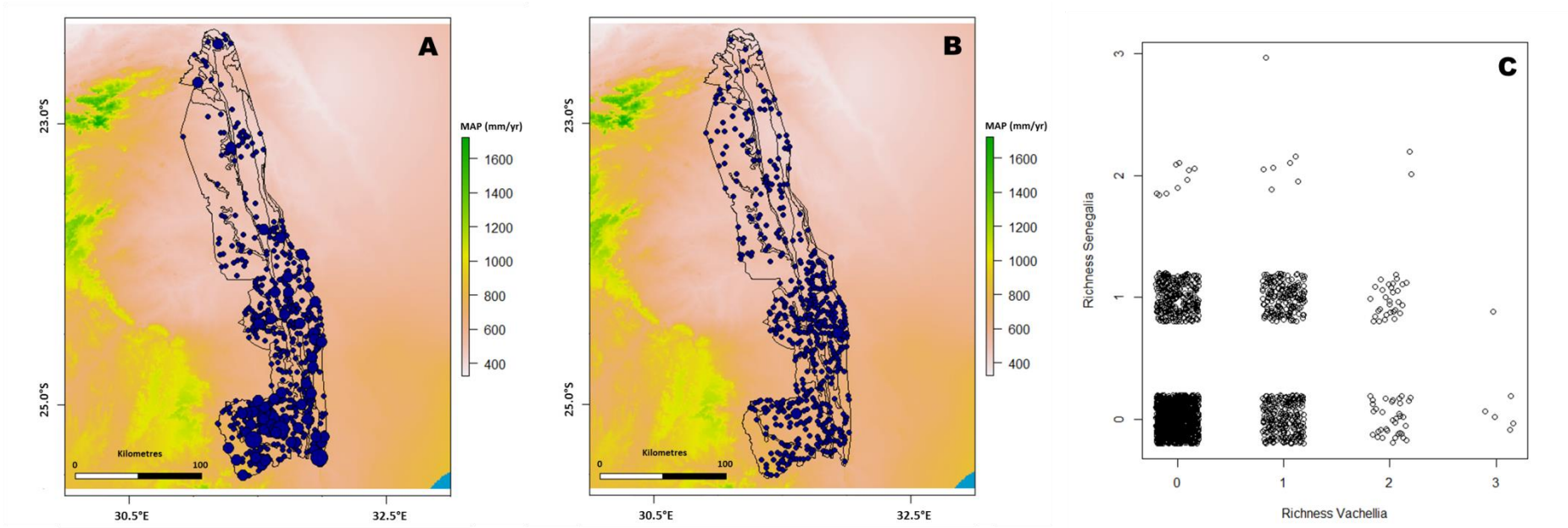

Figure 4: Distribution and richness of *Vachellia* (**A**) and *Senegalia* (**B**) across the Kruger National Park and the correlation of their richness in QDS (**C**). Sizes of points are proportional to the number of species found in 20 X 20 m plots (**A** & **B**). Background layer shows Mean Annual Precipitation (MAP) at 30 arc-second resolution and light blue tiles are ocean (**A** & **B**).

Table 2: Table showing results of Chi-Square analyses, highlighting a non-significant preference for soil type for *Vachellia*, *Senegalia* and their co-occurrence within 20 X 20 m plots distributed across the Kruger National Park.

		Basalt	Granite	Shale	X^2	*P*
Vachellia	Observed	201	292	32	1.400	***0.496***
	Expected	217.9	268.5	38.6		
Senegalia	Observed	235	256	46	2.280	***0.319***
	Expected	222.8	274.7	39.5		
Both	Observed	92	124	17	0.213	***0.900***
	Expected	96.7	119.2	17.1		

unmixed plots with the same richness (Fig 4C). Despite differences in distribution across the Park GLMM and OU analysis revealed no significant differences for any of the chosen habitat variables between genera (Table 1), nor was there a significant association between their occurrence and particular soil types (Table 2). A general lack of significant differences between these genera reflects the high proportion of plots (233 plots = 28.10%) containing species of both. These plots of co-occurrence also show no significant preference for a particular soil type across the Park (Table 2).

Overall phylogenetic structure of plot-scale communities revealed a general trend towards overdispersion, with SES-MPD (mean = 0.26, median = 0.62) and SES-E (mean = 0.18, median = 0.61) being significantly positive (P <0.001; Fig. 5C & D). Moreover, a plot relating $R_{PD\text{-}DO}$ pairwise species co-occurrence index to phylogenetic relatedness appears strongly triangular with a positively sloping hypotenuse. This relationship is significant, the correlation between the response and predictor being significantly greater than expected by chance (rank [obs R^2] = 982, P = 0.018; Fig. 5B). As with QDS assemblages, in contrast to the general pattern of phylogenetic overdispersion, some plots were identified with uncharacteristically strong clustering (large negative values in Fig. 5C & D). While neither SES-E nor SES-MPD analysis identified any individual plot as being significantly clustered, plots with strong clustering were again found to be dominated by species of *Vachellia*. Indeed, where *Vachellia* had high species richness, *Senegalia* was either absent or showed low richness (compare 4A & B vs. Fig. 5A). Strong clustering of *Vachellia* species was most apparent around 25°S latitude (Fig. 5A). By contrast, plots showing evenness were mostly concentrated around 24° 25'S latitude (Fig. 5A). SES-MPD analysis revealed ten plots in the centre and towards the south of the Park that were significantly overdispersed, whereas, SES-E analysis revealed no significantly overdispersed plots. SES-E analysis did, however, identify two significantly clustered plots in the south of the Park where SES-MPD did not.

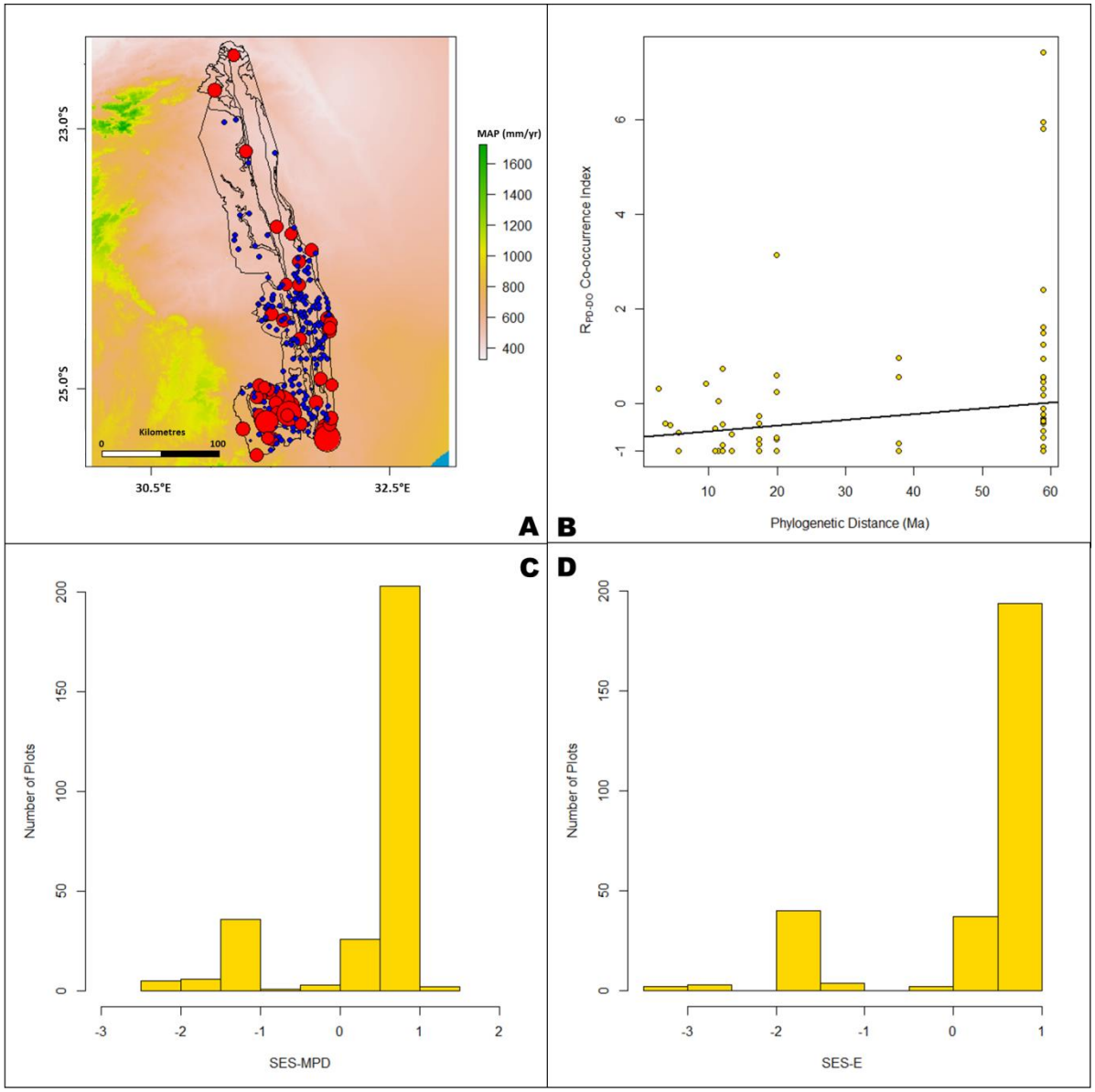

Figure 5: Plot-scale phylogenetic structure of *Vachellia-Senegalia* communities in the Kruger National Park. Distribution of 20 X 20 m plots showing evenness (blue) or clustering (red) where the size of points is proportional to the absolute value of SES-E (**A**). Background layer shows Mean Annual Precipitation (MAP) at 30 arc-second resolution and light blue tiles are ocean (**A**). Basic geology, with an apparent north-west strike, is also annotated, with basalt in the east and granite in the west (**A**). A plot showing a signficant, strongly triangular relationship between R_{PD-DO} co-occurrence index and phylogenetic distance of all species pairs (**B**). Histograms showing positive and significant SES-MPD (**C**) and SES-E (**D**) across all sampled plots.

Discussion

The analyses presented here reveal no significant differences in the climatic associations of *Vachellia* and *Senegalia*, at either the landscape or regional scales. This pattern, coupled with the observation of largely overlapping distributions within southern Africa and the KNP, is suggestive of limited Grinnellian niche differentiation between these genera. Phylogenetic analyses of QDS assemblages and plot-scale communities provides further support in identifying communities of these trees as

being phylogenetically overdispersed, implying that *Vachellia* and *Senegalia* are more likely to co-occur in assemblages and communities than expected by chance. Significant overdispersion in plot-scale communities further suggests that coexistence of these genera at the landscape-regional scale is underpinned by their coexistence at the plot-scale, which I interpret as a consequence of Eltonian niche partitioning.

Plots showing co-occurrence of *Vachellia* and *Senegalia* are largely concentrated around Satara in KNP, demonstrating that this dry region (underlain by both basalt and granite soils) with high herbivore densities provides favourable conditions for species of the two genera to coexist. In principle, the contrasting physical defences of *Vachellia* (spines; Robbertse, 1975a) and *Senegalia* (prickles; Robbertse, 1975a) could facilitate plot-scale coexistence if these defences meant that the two genera were targeted by specialist herbivores, or if generalist herbivores maintained tree densities independently by switching browsing behaviour between the two genera (Chesson, 2000). Both genera are, however, targeted by the same generalist mammalian herbivores, limiting the possibility that differences in physical defence enables their coexistence (Cooper and Owen-Smith, 1986).

The low annual rainfall of 547 mm around Satara, is expected to intensify edaphic competition both between trees and between trees and grasses (Sankaran *et al*., 2005; Wiegand *et al*., 2005; February *et al*., 2013b; Ward *et al*., 2013). This is important given evidence of significant competition for resources between *Vachellia-Senegalia* species (Smith and Walker, 1983; Smith and Goodman, 1986; Shackleton, 2002) and between these species and the grass swards within which they grow (Knoop and Walker, 1985; Cramer *et al*., 2007, 2010, 2012; Riginos, 2009; February *et al*., 2013b). Taken together, these studies suggest that coexistence between *Vachellia* and *Senegalia* in this region might be facilitated by the avoidance of direct competition for edaphic resources. Such coexistence may be enabled through resource partitioning, differences in growth rate in response to fluctuating resources or from differences in establishment in time and/or space (Chesson, 2000).

Vachellia and *Senegalia* may thus separate out water-use by rooting at different depths and being more reliant on either ground or rainwater for growth (February *et al.*, 2007). These genera may also separate in the production of new leaves and germination, however, these biological processes are inextricably tied to the start of the growing season, making such separation unlikely as studies have demonstrated that most savanna tree species leaf out and germinate just before or synchronous with the rains (Coe and Coe, 1987; February and Higgins, 2016). Competition may, however, be avoided through a grass-mediated storage effect (Chesson, 2000), given that grass competition is usually strong during seedling establishment (Scholes and Archer, 1997; Davis *et al*., 1998, 1999;

Riginos, 2009; Cramer *et al*., 2010, 2012) but highly variable in space and time at the plot scale (Chidumayo, 1997; Scholes and Archer, 1997; Jurena and Archer, 2003; Grant and Scholes, 2006; Zambatis *et al.*, 2006; Wakeling *et al.*, 2014). For example, given the longevity of adult trees (storage), if *Vachellia* seedlings were better at establishing when grass biomass was reduced and *Senegalia* seedlings were better at establishing when grass biomass was higher, coexistence of the two genera might be facilitated by differences in establishment time and/or situation (Chesson, 2000).

Strong phylogenetic clustering in certain areas where *Vachellia* species richness is high relative to that of *Senegalia* suggests the tolerance of a much broader suite of habitats for *Vachellia* species and the dominance of this genus where *Senegalia* is absent. *Senegalia*, conversely, does not reflect this pattern, suggesting this genus has a more restricted range of suitable habitats. . It is likely, then, that the environmental variables selected in this study may not have effectively captured the abiotic niches of the two genera. For instance the deep sands found in the Kalahari may underpin the dominance of *Vachellia* in this region, but this edaphic variable was not included in this study. Broadly, my results suggest that Grinnellian niche differentiation is largely restricted to the southern Kalahari, northern parts of the escarpment and the wetter (southern) parts of KNP where *Vachellia* diversity shows clustering. While the southern Kalahari boasts a wide variety of species of *Vachellia*, *S. mellifera* is typically the only *Senegalia* species found in this arid region (Palgrave and Palgrave, 2002). The northern edge of the escarpment is also home to many ecologically diverse *Vachellia* species, with some growing as tall trees, others as shrubs and some even growing in montane forests (Palgrave and Palgrave, 2002). By contrast, *Senegalia* presence is limited to just a few species in this area, all of which grow as tall trees in open grassland (e.g. *S. caffra*, *S. galpinii* and *S. burkei*; Palgrave and Palgrave 2002). In the southern KNP there is a similar pattern in that, while the *Vachellia* species are ecologically diverse, *S. nigrescens* is the only *Senegalia* species typically found in this part of the park (pers. obs.). Overall, the regions with the highest *Vachellia* species richness are at the habitat extremes for fine-leaved savanna trees, and these regions are largely devoid of *Senegalia* species. The high species richness of *Vachellia* in these regions may reflect the wider diversity of ecological strategies employed by this lineage, compared to *Senegalia*, this perhaps being facilitated by its polyploid karyotype (Ross, 1981) and/or its six million year head-start on *Senegalia* as a savanna lineage (Bouchenak-Khelladi *et al.*, 2010).

Interestingly, QDS-scale species assemblages were also observed to be significantly overdispersed. This result is surprising given that, as the spatial scale of analysis increases, increased habitat filtering and biogeographic constraints usually lead to significant phylogenetic clustering between closely

related species (Webb *et al.*, 2002; Cavender-Bares *et al.*, 2009). Observation of coexistence between *Vachellia* and *Senegalia* at the plot-scale, however, suggests that this community-level pattern may trickle up to influence the composition of landscape-to-regional level species assemblages. If the ancestral species of savanna-*Senegalia* evolved a mechanism of Eltonian niche partitioning with already present *Vachellia* when transitioning into the African savanna, the subsequent evolution of this system may have spurred co-diversification between these lineages. Thus, understanding the high-order pattern observed in QDS may depend on understanding plot-scale niche partitioning between *Vachellia* and *Senegalia*.

In conclusion, I suggest that the general pattern of *Vachellia* and *Senegalia* coexistence at the plot-scale in the central parts of the KNP, demonstrates the partitioning of an Eltonian niche. Coexistence of the two genera is also apparent, at the QDS-scale, suggesting that such niche partitioning is a general pattern in southern African savannas. I propose that plot-scale coexistence is enabled either by the avoidance of direct resource competition, through resource partitioning and/or through the evolution of differences in seedling establishment strategy. To address these alternatives, I conducted a large glasshouse experiment using seven species from each genus to determine the nature of functional divergence and differences in seedling survival during establishment among grasses.

Chapter 3:

Functional divergence between *Vachellia* and *Senegalia* may underpin difference in invasiveness and niche partitioning

Introduction

Plant communities become susceptible to invasion when there is a surplus of light, water and nutrients (Davis *et al*., 2000). This situation arises when disturbance reduces the groundcover of vegetation or when the rate at which a particular resource is generated exceeds the rate at which the vegetation exploits it (Davis *et al*., 2000). In tropical rainforests, the primary determinant of plant invasion is the surplus of light that becomes available in tree-fall gaps (Davis *et al*., 2000). In savannas, however, light is not a primary limiting factor for tree recruitment (Charles-Dominique *et al*., 2015a). Rather, tree recruitment is usually limited at the seedling and juvenile stages by competition with grasses for underground resources (Van Auken and Bush, 1990; Bush and Van Auken, 1995; Davis *et al*., 1998, 1999; Jurena and Archer, 2003; Riginos, 2009), with juvenile trees exploiting the same soil horizons as those exploited by hyper-competitive grasses (February and Higgins, 2010; February *et al*., 2013b; Kulmatiski and Beard, 2013).

It has recently been demonstrated that increasing the amount of available water produces an increase in grass productivity but no change in juvenile tree biomass (February *et al*., 2013a). It is, however, not only competition for water that limits tree seedling and juvenile growth among grasses as, these two life history stages are also strongly limited by competition for nitrogen (Cramer *et al*., 2010, 2012), which is only found in the shallow (< 20 cm) soil layers (February and Higgins, 2010; February *et al.*, 2011). To reach adult size-classes successfully, therefore, juvenile trees require gaps in the root mat of grasses (Bush and Van Auken, 1995; Jurena and Archer, 2003; Wakeling *et al.*, 2014). Successful establishment of seedlings into juvenile size-classes, however, is suggested only to occur in grassy ecosystems following disturbance, which reduces grass biomass (Davis *et al*., 1999; Davis *et al*., 2000). In savannas, fire, herbivory and drought provide natural disturbances of the grass layer which, in turn, provide tree seedlings with safe-sites to establish as juveniles (Frost *et al.*, 1986; Scholes and Archer, 1997). Following establishment, juveniles may then persist for several decades trapped in non-reproductive size-classes by fire and herbivory until they release into reproductive adult size-classes above the height of flames and herbivores (Bond and van Wilgen, 1996; Trollope *et al*., 1998; Higgins *et al*., 2000; Wakeling *et al*., 2011; Sankaran *et al*., 2013; Staver and Bond, 2014).

Successful seedling establishment among grasses is, therefore, a critical hurdle facing tree recruitment and invasion in savannas (Brown *et al*., 1998; Davis *et al*., 1998).

Despite the difficulty facing tree recruitment in grassy ecosystems, tree densities and ranges are currently increasing (Stevens *et al.*, 2014, 2017). This phenomenon, termed bush encroachment, poses one of the greatest threats to savannas because it is not fully understood, often overlooked and the cause of an enormous loss of biodiversity (Ward, 2005; Ratajczak *et al*., 2012; O'Connor *et al*., 2014; Nackley *et al*., 2017). Some tree species are, however, more vigorously invasive than are others. Of the eight most invasive species identified in southern Africa, by O'Connor *et al*. (2014), four are species of *Vachellia* and only one is a species of *Senegalia*. Moreover, these authors found that of 18 sites investigated, 90% were encroached by species of *Vachellia* (O'Connor *et al*., 2014). In Chapter 2, I report a general pattern of greater-than-expected levels of coexistence between these two genera at the plot-scale in savannas. Given that coexistence at this scale is normally enabled by the mediation of negative biotic filters, specifically direct resource competition (MacArthur and Levins, 1967; Chesson, 2000; Webb *et al.*, 2002; Cavender-Bares *et al.*, 2009), I suggested that *Vachellia* and *Senegalia* avoid direct resource competition and coexist through Eltonian niche partitioning (Whittaker *et al*., 1973; Soberón, 2007).

In line with this prediction, I highlighted that resource competition between *Vachellia* and *Senegalia* is most likely to be avoided if they are rooted at different depths in the soil profile and so utilise either ground or rainwater respectively (February and Higgins, 2016). This mechanism of competition avoidance seems most applicable since, in savannas, tree growth (February and Higgins, 2016) and germination (Coe and Coe, 1987) are tied to the same time of the year for different species. The inexorable synchronicity of these processes thus limits the possibility that species can coexist by responding differently to fluctuating resources (relative non-linearity; Chesson, 2000; Adler *et al*., 2013) or by differences in the timing of seedling establishment (temporal storage effect; Chesson, 2000; Adler *et al*., 2013). Furthermore, the ecological responsiveness of grasses in savannas (February *et al.*, 2013) means that nutrient-rich patches are unlikely to be stable through time.That grasses can draw down resources in nutrient-rich patches faster than the time it takes for trees to mature, means that the classical coexistence model of spatial heterogeneity (Chesson, 2000; Adler *et al*., 2013) is unlikley to apply, given its primary assumption that spatial differences in resources are stable over the lifetime of the plants coexisting.

The patchiness (variability) of grass biomass at the plot scale (Chidumayo, 1997; Scholes and Archer, 1997; Jurena and Archer, 2003; Grant and Scholes, 2006; Zambatis *et al.*, 2006; Wakeling *et al.*, 2014) may, however, support a form of non-classical spatial coexistence where, resource use in trees

is primarily directed towards grass-free vs. grassy patches which are moving in space and time. Coexistence by such a mechanism suggests that tree species would have different competitiveness among grasses, which could result in the differential survival of their seedlings in the grass layer. Differential seedling survival followed by the non-classical spatial coexistence model highlighted above, could strongly support species coexistence through a grass-mediated storage effect where: seedling establishment is restricted to different times/places in the grass layer and long-lived adults persist (storage) through unfavourable times for establishment (Chesson, 2000; Adler *et al.*, 2013). In the context of this study, if *Vachellia* is more successful at establishing as seedlings when grass biomass is reduced (i.e. after disturbance), compared to *Senegalia*, it could, therefore, not only explain how they coexist in plots but also why *Vachellia* is observed to be relatively more invasive in grassy ecosystems. This makes differences in seedling establishment strategies among grasses important to determine between these genera as, control of invasion by species of *Vachellia* may thus require a different management approach from that of *Senegalia*.

In *Vachellia*, increased seedling establishment success (competitiveness) in the absence of grasses may reflect an avoidance strategy, allowing seedlings to occupy large gaps in the grass layer and/or quickly accumulate resources in the absence of grass competition. Species of *Senegalia*, conversely, may have a tolerance strategy among grasses which increases seedling competitiveness when establishing in smaller gaps among grasses, implying an increased ability to compete directly with grasses. To facilitate an avoidance strategy, species of *Vachellia* may produce lighter and more numerous seeds which promote dispersal into large (low-stress) gaps in the grass layer (Muller-Landau, 2010; Adler *et al.*, 2013). Such a strategy is also facilitated by the production of more-acquisitive (low-leaf mass per area) leaves (Ryser, 1996; Poorter *et al.*, 2009), and greater investment in shallow (<20 cm) and more acquisitive (high-length per mass) roots (Ryser, 1996; Eissenstat 2000; Eissenstat and Volder, 2005; Ryser 2006). In contrast to *Vachellia*, *Senegalia* may better tolerate early grass competition by producing fewer, heavier and less numerous seeds, which increase seedling survival in small (high-stress) gaps in the grass layer (Muller-Landau, 2010; Adler *et al.*, 2013). Tolerance may be facilitated by increased investment in root nodulation associated with symbiotic nitrogen fixation (Cramer *et al*., 2007, 2010, 2012), greater investment in more conservative roots (Ryser, 1996; Eissenstat 2000; Eissenstat and Volder, 2005; Ryser 2006), and the production of leaves with higher mass per area (Ryser, 1996; Poorter *et al.,* 2009). A tolerance strategy would also be enabled by rapid taproot elongation, which is suggested to mitigate early competition with grasses (Brown and Archer, 1990; Bragg, Knapp and Briggs, 1993; Weltzin and McPherson, 1997; Wilson and Witkowski, 1998; Kambatuku *et al*., 2013; Lewis and February, unpublished) and perhaps the development of relatively more fine (<2 mm diameter) roots below

the grass-root layer (>20 cm). These two seedling strategies broadly separate out along the well-understood trade-off axis of rapid resource acquisition with investment in short-lived tissues (*r*-selected, acquisitive) relative to increased efficiency gained with investment in well protected/long-lived tissues (*K*-selected, conservative) (Diaz *et al.*, 2004). The hypothesised differences in seedling establishment strategy among grasses between *Vachellia* and *Senegalia* are illustrated in Figure 1. Differences in life-history strategy between these genera may, however, also work in concert to reinforce competition avoidance through differences in water sourcing. The conservative tissues expected for *Senegalia* may better utilise the deeper, more nutrient poor, soil horizons when sourcing groundwater, whereas, the acquisitive tissues expected for *Vachellia*, could better exploit the shallow, more nutrient rich, soil horizons when sourcing surface water.

Here I test the hypothesis that functional differences during seedling establishment determine Eltonian niche partitioning between *Vachellia* and *Senegalia* in savannas. Specifically, I hypothesise that these genera separate in water-use by differentiation in their rooting systems, enabling access to different water sources (surface vs. groundwater; February *et al.*, 2007). Alternatively, I hypothesise that these genera are different in their seedling establishment strategy among grasses,

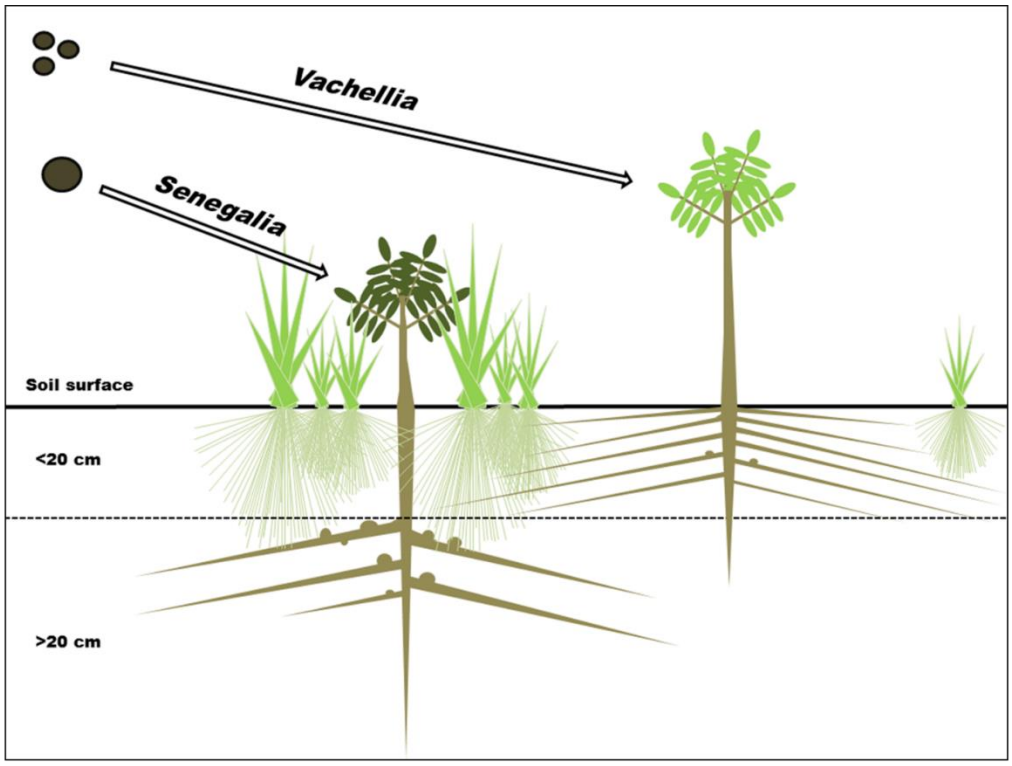

Figure 1: Illustrated combination of seed and seeling trade-offs enabling plot-scale coexistence between *Vachellia* and *Senegalia* in the variable savanna grass layer. Highlighting that while *Vachellia* is tall, shallowly-rooted and sensitive to grass competition, *Senegalia* is short, deeply-rooted and tolerant of grass competition.

with *Vachellia* showing an avoidance strategy and *Senegalia* showing a tolerance strategy, which may or may not work to reinforce the separation of water sources between them. To evaluate these alternatives, I compare seed and seedling traits of seven *Vachellia* and seven *Senegalia* species grown in pots in a glasshouse, both in the presence and absence of competition from grasses. I do this over a period of 17 months incorporating two growing seasons separated by a dry season.

Methods

The study was conducted in the glasshouse of the Department of Biological Sciences at the University of Cape Town between 25 February 2015 and 8 July 2016. For the experiment, nine individuals of each of seven *Vachellia* and seven *Senegalia* species were grown in pots, both with and without grasses (63 seedlings per treatment per species). Species were selected to represent mesic, xeric and intermediate savannas, inferred from habitat type as set out in Palgrave and Palgrave (2002), to cover the wide ecological range occupied by these genera in savannas (Table 1).

Seed traits

For ten seeds of each species, obtained from the indigenous seed distributor Silverhill Seeds (Kenilworth, South Africa), the total weight was determined using a balance precise to 0.1 mg (Shimadzu AUW220D, Kyoto, Japan), following which the testa, cotyledon and embryo were separated (see Fig. 1, Supp. 1) and weighed separately. Using digital calipers, testa thickness was then determined as the average of the adaxial and abaxial thickness of the seed coat. Surface area to volume ratios were also calculated for each seed using measurements of seed length, thickness and width and the following formulae:

$$Ellipsoid\ volume\ = \frac{1}{4} \times \pi \times length \times thickness \times width$$

$$Ellipsoid\ surface\ area = 4 \times \pi \times (\frac{[width \times length]^{1.6} \times [width \times thickness]^{1.6} \times [length \times thickness]^{1.6}}{3})^{\frac{1}{1.6}}$$

Experimental setup and plant propagation

Germination was induced in three ways: (1) for some species, it was sufficient to soak seeds in an aerated water bath overnight; (2) for others a warm soaking (30 °C) was required; and (3) for the remaining species, it was first necessary to clip the testa and then soak in warm water overnight (Table 1). After germination, for the no-grass treatment, nine individuals of each species were planted into separate 20 x 15 cm (height x diameter) pots, each containing a 40:60 mix (in a cement mixer) of potting soil (Master Potting Mix, Master Organics, South Africa) to swimming pool filter sand (14/30 silica sand, Cape Silica Suppliers, South Africa) on top of a ± 2 cm layer of drainage chips

Table 1: Differences in the technique used for the germination of species of *Vachellia* and *Senegalia* in the glasshouse experiment, relative to their preferred savanna type, growth form and pod type.

Species	Habitat Type	Growth Form	Pod Type	Germination Technique
S. ataxacantha (DC.) Kyal. & Boatwr.	Mesic	Shrub to small tree	Dehiscent	Cold soaking
S. caffra (Thunb.) P.J.H.Hurter & Mabb.	Mesic	Tree	Dehiscent	Cold soaking
S. erubescens (Welw. ex Oliv.) Kyal. & Boatwr.	Xeric	Shrub to small tree	Dehiscent	Cold soaking
S. mellifera (Vahl) L.A.Silva & J.Freitas	Xeric	Shrub to small tree	Dehiscent	Cold soaking
S. montis-usti (Merxm. & A.Schreib.) Kyal. & Boatwr.	Xeric	Shrub to small tree	Dehiscent	Cold soaking
S. nigrescens (Oliv.) P.J.H.Hurter	Intermediate	Tree	Dehiscent	Cold soaking
S. senegal Britton	Intermediate	Shrub to small tree	Dehiscent	Warm soaking
V. erioloba (E.Mey.) Seigler & Ebinger	Xeric	Shrub to small tree	Indehiscent	Clipped testa and warm soaking
V. haematoxylon (Willd.) Seigler & Ebinger	Xeric	Shrub to small tree	Indehiscent	Clipped testa and warm soaking
V. karroo (Hayne) Banfi & Galasso	Mesic	Tree	Dehiscent	Clipped testa and warm soaking
V. robusta (Burch.) Kyal. & Boatwr.	Intermediate	Tree	Dehiscent	Clipped testa and warm soaking
V. nilotica (L.) P.J.H.Hurter & Mabb.	Mesic	Tree	Indehiscent	Clipped testa and warm soaking
V. sieberiana (DC.) Kyal. & Boatwr.	Mesic	Tree	Indehiscent	Clipped testa and warm soaking
V. tortilis (Forssk.) P.J.H.Hurter & Mabb.	Intermediate	Tree	Indehiscent	Clipped testa and warm soaking

(crushed stone). For the with-grass treatment, germinated seedlings were similarly treated, but three (2 x 5 cm) plugs (containing several individuals matted together) and six (individually separated) individuals of *Pennisetum clandestinum* Hochst. ex Chiov., a common and vigorous East African C_4 savanna grass species, were planted in each pot around the tree seedling in the centre. Grasses were initially clipped to 5 cm above soil level and subsequently regularly trimmed to below 15 cm to limit the possibility of light competition between grasses and tree seedlings during the experiment. All pots were randomly allocated to a trolley and position on the trolley using a random number generator (Microsoft Excel, 2010). The trolleys were then rotated fortnightly for the duration of the experiment. On day 0 (25/02/2015), after all experimental seedlings had been planted, all pots were supplied with a small once-off dose of 18 g of slow-release fertiliser (Vita Veg 6:3:4 (16), Talborne Organics, South Africa) and inoculated with both granite- and basalt-derived soils, collected in

the KNP the previous week, to ensure the presence of rhizobia species necessary for the development of nitrogen-fixing nodules. Seedlings that had died prior to day 85 (22/05/2015) were replaced with individuals which had been germinated at the same time as the experimental plants and grown separately in stock seedling trays.

To compare the nutrient concentrations of experimental soils with soils sampled from the field in the KNP, five randomly selected soil samples from each treatment (with-grass and no-grass) were sent for analysis of plant available nitrogen and phosphorus to the Institute for Plant Sciences Department of Agriculture, Elsenburg, South Africa. For this analysis, approximately 50 g of soil was collected from just below the surface and dried at 70 °C for 48 hours in an oven (Scientific Series 2000, model 278, South Africa). Dried samples were then milled using a Wiley mill with a 0.5 mm mesh. The concentration of phosphorus was determined using Inductively Coupled Plasma Optical Emission Spectrometry (iCAP 7000 Plus Series ICP-OES, ThermoFisher Scientific, MA, USA) on 1% citric acid soil extracts (Thompson, 1995). For determination of nitrogen, Kjeldahl digests were performed on soil samples that were first heated with sulphuric acid and then distilled using sodium hydroxide, the concentration of NH_4-N then being determined using back-titration (Kjeldahl, 1883). Experimental soil nutrient concentrations were then compared with values obtained in the Kruger National Park by Craine *et al.* (2008) for nitrogen and Du Toit *et al*. (1990) for phosphorus.

To simulate rainfall seasonality in savanna, watering was incrementally reduced towards the end of the dry season (September) after which, watering was again increased to simulate the return of the wet season. For the first two weeks the experiment was automatically watered once a day for 10 min (226 ± 98 ml per pot) using an overhead sprinkler system, following which the same amount of water was supplied every second day. From day 85 (22/05/2015), watering was reduced to 25 min once a week and from day 177 (24/08/2015) to 15 min per week. From day 184 (31/08/2015) watering was reduced further, to 5 min once per week, to simulate dry season conditions in the field. From day 225 (12/10/2015) watering was increased to 5 min twice a week, from day 232 (19/10/2015) to 10 min twice a week, and from day 252 (26/10/2015) to 15 min twice a week. Finally, watering was increased to 20 mins twice a week from day 266 (23/11/2015), with pots receiving additional water by hand, until saturation, on very hot days for the remainder of the experiment. The watering regime is summarised in Table 1, Supp. 1.

Seedling growth and dry period survival

Shoot height, from the soil surface to the apical meristem, was measured every second month using a steel ruler, while the stem diameter of each seedling was measured directly below the cotyledon

scar using digital Vernier calipers. These data were used to generate growth curves for each species (detailed below) over the duration of the experiment.

The ability of individual species' seedlings to survive the initial dry season was quantified as the proportion of seedlings resprouting successfully following the simulated dry season between days 184 and 225. Despite being only six months old, all species in this experiment showed some ability to resprout. In order to capture the flushing response of seedlings (cf. Rossatto *et al*., 2009), I determined the Fournier Intensity Index (Fournier, 1974) of each individual for 22 weeks following the end of the dry period. For this purpose, canopies were grouped into five categories of leaf flush: 0 = canopies with 0% new leaves, 1 = 1-25% new leaves, 2 = 26-50% new leaves, 3 = 51-75% new leaves and 4 = 76-100% new leaves. These data were also used to determine which seedlings had died during the dry period (category 0 at 22 weeks after the start of the new wet season). These data were later used to calculate a percentage survival for each species in both treatments. Additionally, in the with-grass treatment, grass survival was also determined each week based on the presence or absence of new grass growth. These data were later used to determine whether tree seedlings had escaped grass competition by surviving the dry period when neighbouring grasses did not. These data were then used to calculate a percentage escape for each species in the with-grass treatment.

Harvesting and trait measurement

Harvesting was initiated on day 394 and ended on day 491, with the mean age of individuals at harvest being 442.2 ± 23.3 days for *Vachellia* and 451.0 ± 28.1 days for *Senegalia*. Following harvest, each individual was divided into the above-ground (leaves, stem and physical defences) and below-ground (roots and nodules) fractions with each part being bagged and dried separately at 60 °C for 72 hours prior to weighing for dry mass.

Aboveground measurements

Leaf Mass per Area

Leaf mass per area was determined on three fully-expanded leaves. Each leaf was separated into leaflets, petiole, rachis and rachilla and photographed against a white background along with a 1 cm^2 square of graph paper at a fixed focal length using a Canon EOS 600D camera (Canon Inc., Japan). The photos were then manipulated into 8 bit, black and white, negatives using the Fiji (ImageJ) image processing package version 1.51a before calculating leaflet area using the same software analyse particles function (Schindelin *et al.*, 2015). Leaflets, rachis and rachilla were then bagged and dried separately at 60 °C for 72 hours. The leaf mass per area of each individual was determined by

dividing the total oven dry mass of leaflets (excluding rachis and rachilla) of the three selected leaves by the one sided-area of their leaflets when fresh (Cornelissen *et al.*, 2003). Both fresh and dry weights were recorded using a balance precise to 1 mg (Shimadzu AUW220, Kyoto, Japan). While Poorter *et al.* (2009) have highlighted the importance of determining the individual leaf components which underpin leaf mass per area, namely leaf volume per area (≈ thickness) and leaf tissue density, leaf volume per area was not calculated in this study.

Wood density

The longest stem of each tree was cut at 5 cm and 7 cm above the soil surface in order to separate a 2 cm section for wood density. Using digital Vernier calipers, stem diameter at 5 cm was determined before and after the removing bark with the difference between the two used to calculate both bark thickness and bark growth rate (absolute bark thickness divided by the age of the individual at harvest). Absolute bark thickness was then divided by stem diameter for relative bark thickness (Lawes *et al*., 2013). To determine wood density the 2 cm section of wood (with the bark removed) was submerged in a beaker of water on a balance precise to 1 mg (Shimadzu AUW220, Kyoto, Japan), the amount of force needed to submerge the wood just below the surface of water being used as a measure of volume (Chave, 2005). Volume was then divided by the dry mass of wood to calculate wood density (Chave, 2005). Unfortunately it was not possible to determine wood density for several with-grass individuals as their wood volumes were too small to be measurable.

Belowground measurements

Each tree was removed from the pot with the soil and the root system delicately teased apart by hand in a plastic bucket filled with warm water to minimise root breakage. Root length was then measured as the length of the longest root from the root-shoot interface using a 1 m long steel ruler. The untangled roots were then suspended and divided into three fractions: (i) roots situated within 20 cm of the root-shoot interface (shallow roots), (ii) roots situated more than 20 cm from the root-shoot interface (deep roots), and (iii) nodules. The separation of roots into shallow and deep fractions was done to determine whether root biomass was differentially invested down the soil profile between genera. The threshold of 20 cm was chosen on account of the observations that most nitrogen (February and Higgins, 2010; February *et al*., 2013b) and grass activity (Kulmatiski *et al.*, 2010; Kulmatiski and Beard, 2013) is associated with soils shallower than 20 cm in savannas. The fresh weights of the three root fractions were determined using a balance precise to 1 mg (Shimadzu AUW220, Kyoto, Japan). In each root fraction, total root length, volume, diameter, and length of the fine (<2 mm) and coarse (<2 mm) root fraction was determined using WinRHIZO software (version 2013a, Regent Instruments Inc., Canada). After scanning, the roots were dried at 60 $^{\circ}$C for 72 hours to determine specific root length (SRL) and root tissue density (RTD), using a balance precise to 1 mg

(Shimadzu AUW220, Kyoto, Japan) to measure dry weight. These root traits were measured because of their important influence over below-ground resource-use (Ryser, 1996; Eissenstat and Volder, 2005; Ryser, 2006).

Statistical analysis

Differences in the concentrations of nitrogen and phosphorus between the experimental soils and field-sampled soils from the KNP were determined using two-tailed Wilcoxon Ranked Sum Tests in R (R Core Team, 2016). For this analysis, the reported values from the field were tested independently against values for each of the experimental soil types (pre-experimental, with grass and no grass). Following Bonferroni correction, no significant differences were observed in either nitrogen or phosphorus between reported values from field-sampled soils and the values obtained for the experimental soils.

To test for significant trait differences between *Vachellia* and *Senegalia*, I used a generalized linear mixed effects model (GLMM) treating genus as a fixed effect and species as a random effect nested within genus. For glasshouse trait data, treatment was included as a further fixed effect and, in the event of a significant interaction between treatment and genus, separate mixed models were run for each genus and treatment to test for significant effects and for the purpose of fitting curves. For time series data, GLMM model error structure was expanded to include individual to account for the non-independence of repeated measures on the same individual. These GLMM regressions were then used to calculate differences in growth rate between genera for these traits. GLMM's were run using the *lme4* package version 1.1-15 (Bates *et al.*, 2015) in R. Significant differences between genera were assessed using p-values generated by Satterthwaite's degrees of freedom method as implemented in the *lmerTest* package version 3.0 (Kuznetsova *et al*., 2017). For survival and escape data, a t-test was preferred over GLMM for the purpose of assessing significant differences between genera. This test was run using the *t.test()* function in the *stats* package of R (R Core Team, 2016). This was done because differences in these traits were calculated as species means, such that species could not be included as an error term.

To account for phylogenetic non-independence of species, I also fitted Ornstein Uhlenbeck (OU) models of character evolution to assess whether traits were evolving under different selection optima in the two lineages (Hansen, 1997; Butler and King, 2004). OU models were run using species means, with the significance of differences between *Vachellia* and *Senegalia* being determined by comparing the Akaike information criterion (AIC) scores of a single optimum model (OU1) and a two-optimum model (OU2, fitted to *Vachellia* and *Senegalia*). The best model was then selected based on its AIC score. Where OU2 had an AIC score <2 compared to OU1, this was considered as strong

evidence of significant trait divergence between *Vachellia* and *Senegalia* (Burnham and Anderson, 2002). OU models were fitted in the context of the *Mimosoideae* phylogenetic tree generated in Chapter 2, but pruned to include only the experimental species, using the *hansen()* function from the *ouch* package version 2.11-1 (King and Butler, 2004).

For both GLMM and OU analyses, all seed trait data except surface area to volume ratio were log-transformed. For these same analyses all glasshouse traits except wood density, root average diameter, root tissue density and fresh mass of nodules were log-transformed to ensure normality of residuals. For statistical testing of nodule fresh weight, *S. ataxacantha*, *S.mellifera* and *S. nigrescens* were excluded, as these species did not produce nodules under either treatment. Also, these species were not given a value of zero because it was impossible to determine whether (or not) they were capable of nodulating. To ensure normality, percentage survivorship was power-transformed and percentage escape square root-transformed. Transformed data were then used for both t-testing and OU analysis. To ensure GLMM model convergence, for analyses of time-series data, height and stem diameter were both log-transformed as was the time since germination.

Results

Seed traits

There was no significant difference (GLMM, P > 0.05) in seed mass between *Vachellia* (132 ± 23 g) and *Senegalia* (164 ± 49 g), with the OU1 model also being strongly favoured for this trait (AIC_{OU1}-AIC_{OU2} = -1.98) (Table 2). Testa were significantly (GLMM, P < 0.001) thicker in *Vachellia* (0.48 ± 0.07 mm) than in *Senegalia* (0.16 ± 0.02 mm), while seed surface area to volume ratio was significantly greater (GLMM, P < 0.05) in *Vachellia* (1.1 ± 0.2 mm) than in *Senegalia* (1.7 ± 0.2 mm) (Table 2). These differences are corroborated by strong support for the OU2 model (average testa thickness: AIC_{OU1}- AIC_{OU2} = 4.99; surface area to volume ratio: AIC_{OU1}- AIC_{OU2} = 2.16; Table 2).

Table 2: Raw mean (of species means) ± SE values for measured seed traits of *Vachellia* (V) and *Senegalia* (S). A significant difference is shown by *(P<0.05), **(P<0,01), *** (P<0.001) and no significant difference is denoted by NS. The sign of ΔAIC represents whether an OU1 model (negative) or and the OU2 model (positive) is favoured. ΔAIC >2 suggests a strong divergence between these lineages and these values are highlighted in bold.

	Vachellia	*Senegalia*	LME	ΔAIC
Total weight (mg)	132 ± 23	164 ± 49	NS	-1.98
Cotyledon Weight (mg)	61 ± 11	109 ± 34	NS	-1.22
Testa Weight (mg)	69 ± 14	53 ± 15	NS	0.01
Embryo Weight (mg)	2.9 ± 0.4	2.5 ± 0.7	NS	-0.75
Average Testa Thickness (mm)	0.48 ± 0.07	0.16 ± 0.02	**V > S *** **	**4.99**
Surface Area : Volume	1.1 ± 0.2	1.7 ± 0.2	**V < S ***	**2.16**

Seedling growth and survivorship

In the absence of grasses, the mean vertical growth rate of *Vachellia* (mean = 0.99 log[cm]/log[days since germination]; Fig. 2) was 23 % greater than that of *Senegalia* (mean = 0.76 log[cm]/log[days since germination]; Fig. 2), this difference being significant (GLMM, $P < 0.05$; Table 3). This result is corroborated by seedling height data, collected at harvest, which show that *Vachellia* seedlings grew significantly taller (61 ± 6 cm) than *Senegalia* seedlings (37 ± 5 cm) during the course of the experimental period (GLMM, $P < 0.01$; Table 4). This height difference is also revealed by a comparison of OU model fits, which clearly favours the OU2 model (AIC_{OU1}- AIC_{OU2} = 4.3; Table 4). *Vachellia* seedlings also grew significantly taller per unit stem diameter (70 ± 9 cm) than *Senegalia* seedlings in the absence of grasses (40 ± 5 cm; GLMM, $P < 0.01$; Table 4). When grown with grasses, however, there were no significant differences in growth rate, final plant height or height to stem diameter ratio between the two genera (Fig. 2, Table 4). Relative to the no-grass treatment, grass competition significantly reduced (GLMM, $P < 0.001$; Table 3) growth rate in both height and stem diameter in *Vachellia* and *Senegalia* (Fig. 2) and also reduced final plant height and stem diameter at harvest in both genera (GLMM, $P < 0.001$; Table 4).

For the following variables, *Vachellia* and *Senegalia* did not differ significantly under either treatment, though grass competition significantly reduced the values of these traits: total dry mass (GLMM, $P < 0.001$; Table 4), root length (GLMM, $P < 0.5$; Table 4), root average diameter (GLMM, $P < 0.001$; Table 4), fresh mass of nodules (GLMM, $P < 0.001$; Table 4), leaf mass per area (GLMM, $P < 0.001$; Table 4) and percentage survival (GLMM, $P < 0.01$; Table 4). Specific root length, however, showed the opposite pattern in that, while *Vachellia* and *Senegalia* did not differ significantly under either treatment, the presence of grasses produced an overall increase in specific root length (GLMM, $P < 0.001$; Table 4).

Traits identified by GLMM as differing significantly between *Vachellia* and *Senegalia* (viz. plant height and height-to-stem-diameter ratio in the no-grass treatment) generally conformed better to the OU2 model than the OU1 model, indicating divergent selection on these traits between the two genera. Despite GLMM showing no significant difference for root tissue density between genera (Table 4), comparison of OU model fits strongly favoured the OU2 model for plants grown in the presence of grasses (AIC_{OU1}- AIC_{OU2} = 3.7; Table 4), thereby providing strong evidence that the response of this trait to competition was under divergent selection between the two genera. A *post hoc* ANOVA of species means clarified this OU result, showing that a significant difference between

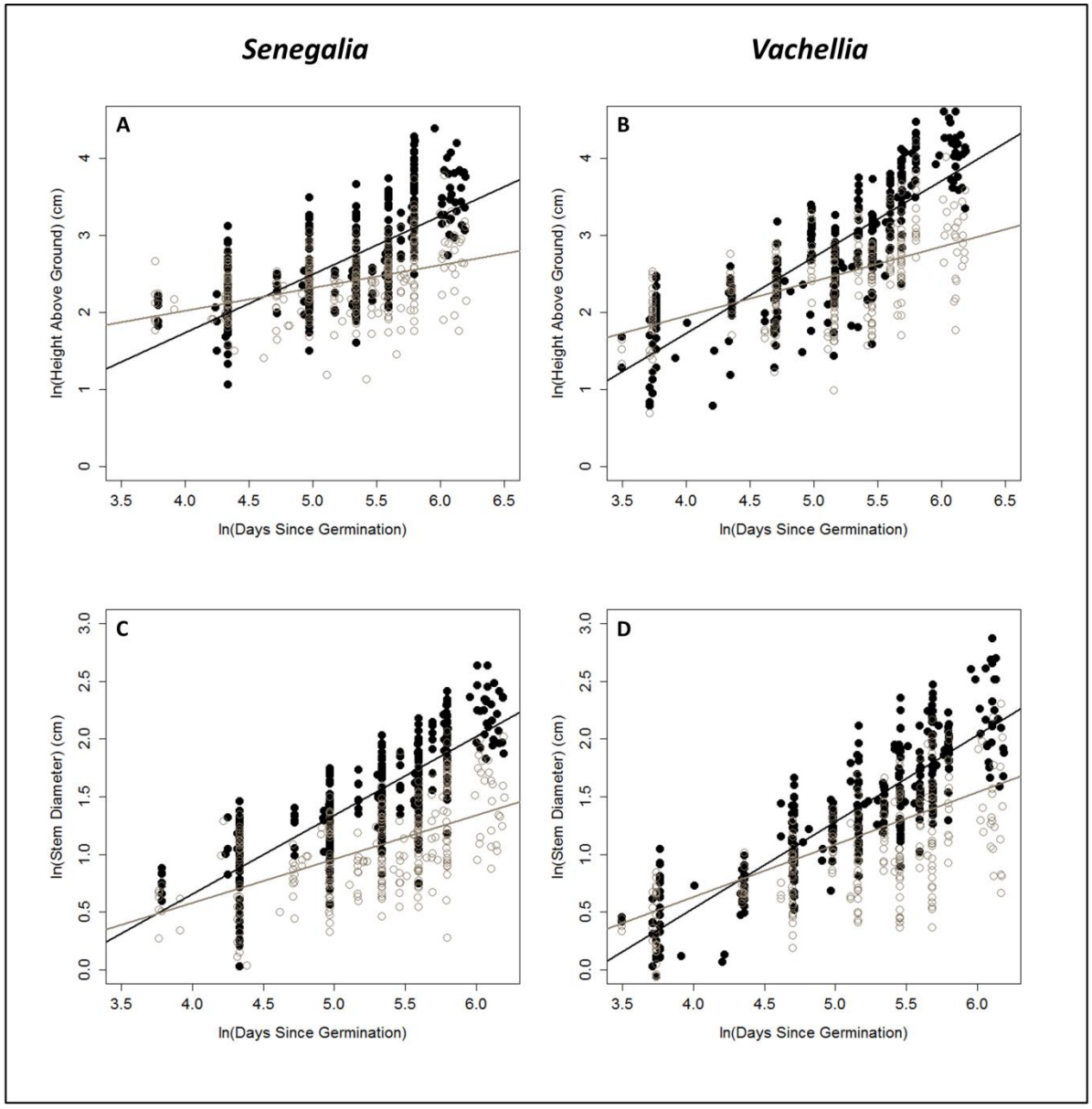

Figure 2: The relationship between days since germination and height above ground for *Senegalia* (**B** & **C**) and *Vachellia* (**B** & **D**) illustrating significantly greater ($P < 0.05$) growth rates for *Vachellia* in the no grass treatment (solid black circles) relative to *Senegalia* (**A** & **B**). Growth rates in height were, however, comparable in the with-grass treatment (open grey circles) between these lineages (**A** & **B**). There was no significant difference in stem diameter growth rate for either treatment between them (**C** and **D**). Lines represent fitted GLMM's.

genera is only observed in the presence of grasses ($ANOVA_{WG}$, $P < 0.01$ & $ANOVA_{NG}$, $P > 0.05$; see Fig. 3). Further support for divergent selection on root tissue density between genera in the presence of grass competition was shown by a test of phylogenetic signal, which was significant in the with-grass treatment but not in the no-grass treatment ($Phylosignal_{WG}$, $P < 0.001$ & $Phylosignal_{NG}$, $P > 0.05$; see Fig. 3). The observed difference in root tissue density between the two genera is a consequence of increased root tissue density in *Senegalia* in the presence of grasses (Fig. 3). Overall, these results

Table 3: Results of GLMM analysis of plant height and stem diameter growth rates between *Vachellia* (V) and *Senegalia* (S) in the with-grass (WG) and no-grass (NG) treatments. Significant difference/interaction is shown by *(P<0.05), **(P<0,01), *** (P<0.001).

Height above ground		
Treatment*time	**NG>WG*** **	**NG: V>S*** WG: NS
Genus*time	**V>S***	**V: NG>WG*** ** **S: NG>WG*** **
Stem diameter		
Treatment*time	**NG>WG*** **	NG: NS WG: NS
Genus*time	NS	

suggest that *Senegalia* species have a root tissue density response to direct grass-root competition, whereas, species of *Vachellia* have none.

Table 4: Raw mean (of species means) ± SE values for *Vachellia* (V) and *Senegalia* (S) after cultivation in two treatments, with (WG) and without grasses (NG). The average age for the individuals at harvest was 442 ± 23 days for *Vachellia* and 451 ± 28 days for *Senegalia*. A significant difference is shown by *(P<0.05), **(P<0,01), *** (P<0.001) and no significant difference is denoted by NS. The sign of ΔAIC represents whether an OU1 model (negative) or and the OU2 model (positive) is favoured. ΔAIC >2 suggests strong divergence between these lineages and these values are highlighted in bold.

	Vachellia		*Senegalia*						
	No Grass	**With Grass**	**No Grass**	**With Grass**	**Interaction**	**Treatment**	**Genus**	**No Grass ΔAIC**	**With Grass ΔAIC**
					Leaf/plant traits				
Height (cm)	61 ± 6	21 ± 4	37 ± 5	17 ± 2	**0.016**	**V: NG > WG *** S: NG > WG *** **	**NG: V > S ** WG: NS**	**4.3**	-1.4
Stem Diameter (cm)	0.95 ± 0.13	0.46 ± 0.08	0.95 ± 0.07	0.46 ± 0.05	0.714	**NG > WG *** **	NS	-2.0	-2.0
Height : Stem Diameter	70 ± 9	52 ± 9	40 ± 5	38 ± 3	**0.017**	**V: NG > WG *** S: NS**	**NG: V > S ** WG: NS**	**3.9**	-0.9
Root : Shoot (Drymass)	1.1 ± 0.2	1.2 ± 0.2	1.3 ± 0.3	1.3 ± 0.4	0.837	NS	NS	-1.7	-1.6
Leaf Mass Area (kg/m²)	0.089 ± 0.015	0.073 ± 0.008	0.078 ± 0.009	0.057 ± 0.005	0.133	**NG > WG *** **	NS	-1.7	0.9
Total Dry Mass (g)	24 ± 3	3.2 ± 0.8	20 ± 2	3.7 ± 0.8	0.107	**NG > WG *** **	NS	-1.2	-1.8
Relative Bark Thickness (%)	37 ± 5	NA	28 ± 3	NA	NA	NA	NS	0.3	NA
Wood Density (g/cm³)	0.80 ± 0.01	NA	0.85 ± 0.02	NA	NA	NA	NS	0.4	NA
					Root traits				
Root Length (cm)	82 ± 12	60 ± 6	77 ± 6	62 ± 6	0.684	**NG > WG *** **	NS	-2.0	-1.9
Root Tissue Density (g/cm³)	0.47 ± 0.01	0.46 ± 0.02	0.51 ± 0.03	0.54 ± 0.01	0.169	NS	NS	-0.4	**3.7**
Root Average Diameter (mm)	0.42 ± 0.01	0.37 ± 0.02	0.41 ± 0.01	0.33 ± 0.01	0.076	**NG > WG *** **	NS	-2.0	0.2
Specific Root Length (cm/g)	659 ± 32	1164 ± 117	756 ± 85	1177 ± 172	0.559	**NG < WG *** **	NS	-1.3	-2.0
Length Fine Roots Shallow : Deep	2.4 ± 0.6	2.8 ± 0.6	3.4 ± 1.2	2.4 ± 0.4	0.142	NS	NS	-1.7	-1.7
Fresh Mass Nodules (g)	3.8 ± 1.6	0.51 ± 0.29	2.0 ± 0.7	0.14 ± 0.13	0.490	**NG > WG *** **	NS	-2.0	-1.6
					Survival and escape				
Percentage Survival (%)	95 ± 3	73 ± 4	98 ± 2	84 ± 5	0.313	**NG > WG ** **	NS	-1.4	1.4
Percentage Escape (%)	NA	3.2 ± 2.0	NA	7.8 ± 3.2	NA	NA	NS	NA	-0.8

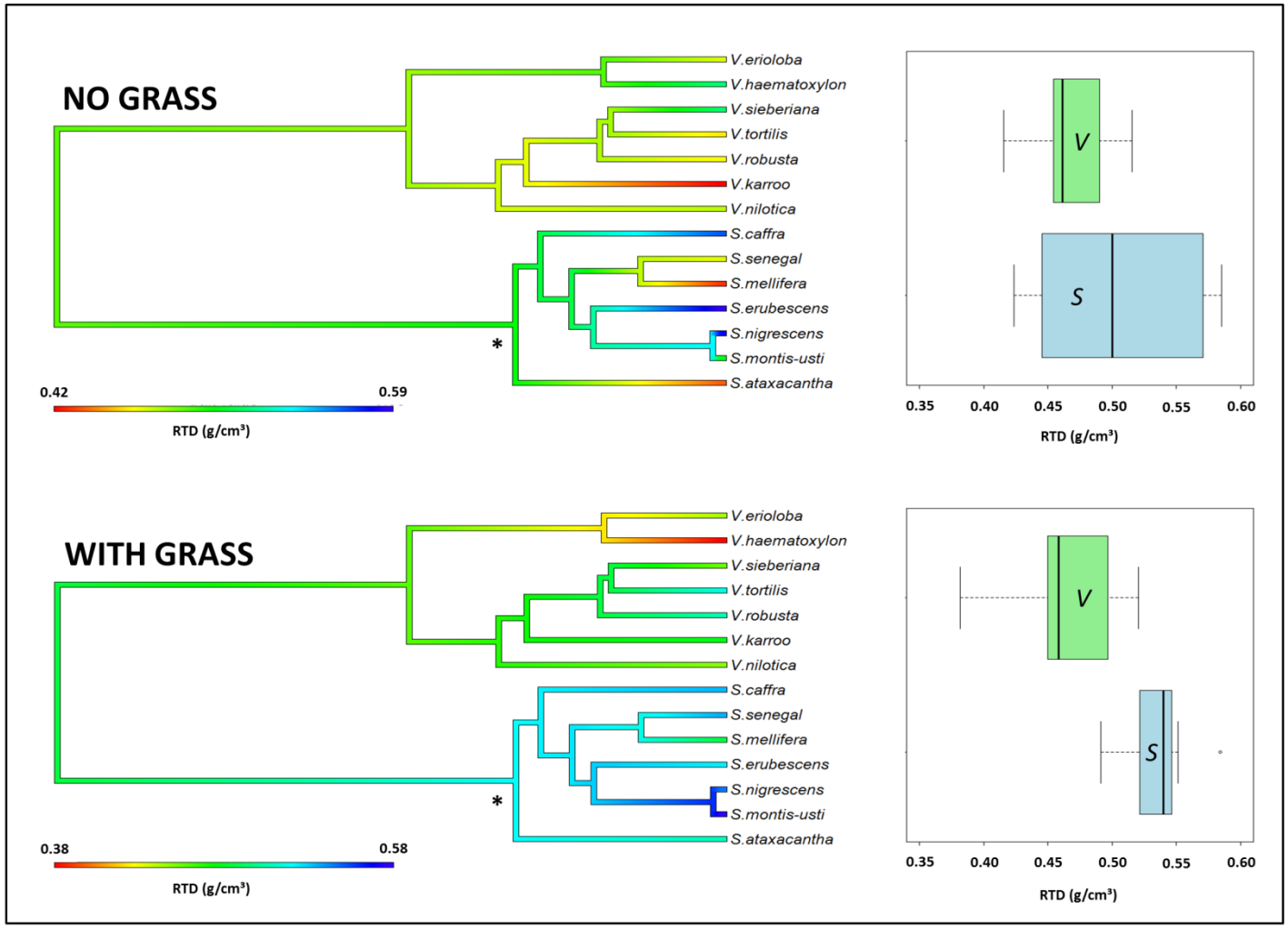

Figure 3: Phylograms (left) and boxplots (right) of root tissue density (RTD) in the no-grass treatment (top) and the with-grass treatment (bottom) illustrating the strong, clade-wide, upregulation of root tissue density by *Senegalia* (S) when in direct root-competition with grasses. This response is highlighted by the colour change at *Senegalia*'s crown node (*), compared to the lack of colour change at *Vachellia*'s crown node. Boxplots illustrate a significant difference in root tissue density between *Vachellia* and *Senegalia* in the with-grass treatment only ($ANOVA_{WG}$, $P < 0.01$).

Discussion

Contrary to my primary hypothesis, my results show no significant difference in either total root length or the distribution of fine roots across a soil depth profile between the two genera. These results, suggest that the plot-scale coexistence of *Vachellia* and *Senegalia* is unlikely to be attributable to differences in the depth at which the two genera obtain water as has been demonstrated for *Philenoptera violacea* and *Colophospermum mopane* (February *et al.*, 2007). While my alternative hypothesis predicted that *Senegalia* seedlings would fare better with direct competition for resources from grasses, my results show that this competition resulted in similarly reduced biomass and increased mortality in both genera. These results suggest that both genera require similar-sized gaps (safe sites) for successful seedling establishment among grasses. This finding is in line with Dohn *et al.* (2017), who demonstrate that savanna tree seedlings and juveniles are strongly aggregated into gaps in the grass layer, despite decreased growth rates.

In explaining coexistence between *Vachellia* and *Senegalia*, my results show significant differences in seed architecture between these two genera. Where the seeds of *Vachellia* have a thicker testa and are more spherical, than that of *Senegalia* which are flatter and more discoid. These differences likely reflect their underlying differences in dispersal strategy, where all species of *Senegalia* are wind-dispersed with dehiscent pods, accounting for the discoid-shape (Coe and Coe, 1987), most species of *Vachellia* are dispersed in the gut of large mammals and have evolved indehiscent pods, with thick-coated seeds, to facilitate this (Ross, 1979; Coe and Coe, 1987). Although some species of *Vachellia* in this study (*V. karroo* and *V. robusta*) are wind-dispersed, the seeds of these species are nonetheless more spherical and thick-coated than those of *Senegalia*, suggesting that these species can be successfully dispersed by large mammals (O'Connor *et al*., 2010) and that they are not as well suited to wind dispersal. Differences in dispersal mode between these genera could thus explain the apparent (but not significant) differences in cotyledon and testa weight between them. Phylogenetic Generalised Least Squares (PGLS) regression (not presented) demonstrated the strong trade-off between these traits and showed that, for any given seed weight, *Vachellia* invests significantly more into its testa.

Conserved differences in dispersal mode between *Vachellia* and *Senegalia* are potentially important for seedling establishment because, wind dispersal is directed towards the centre of gaps, whereas animal dispersal is directed towards gap edges (Schupp *et al.*, 1989; Wenny, 2001). This is because, where animals tend to move along gap edges, wind-dispersed seeds tend to fall in updraft-eddies which form in the hot, bare centres of gaps (Schupp *et al.*, 1989; Wenny, 2001). Despite these

differences, however, savanna vegetation structure is relatively uniform (Charles-Dominique *et al*., 2015a), with the consequence being that the strength of eddies is diminished and gap edges poorly defined (Wenny, 2001). The result is that seeds from both genera are evenly distributed across the landscape reducing the possibility that differences in dispersal between *Vachellia* and *Senegalia* have significant influences on seedling establishment. Differences in investment into cotyledon vs. testa weight may, however, mean that, while germinating in the same places, *Senegalia* seedlings are more competitive for a given seed weight since they invest relatively more into their cotyledons (Muller-Landau, 2010) and less into testa, which could slow the speed of germination (Coe & Coe, 1987).

While both genera may indeed show reduced growth rates with competition from grasses my results also show that *Vachellia* grew significantly taller than *Senegalia* with no competition from grasses. These faster growth rates without competition from grasses would suggest that *Vachellia* species are adapted to rapidly establish from seedling to juvenile and finally to reproductive size classes when grass biomass is reduced (Trollope, 1984; Bond and van Wilgen, 1996; Higgins *et al*., 2007; Sankaran *et al*., 2013; Staver and Bond, 2014). While *Vachellia* may grow taller than *Senegalia* when grass biomass is reduced there is a significant increase in root tissue density for *Senegalia* but not for *Vachellia*, with competition from grasses. Several studies have now demonstrated than an increase in root tissue density generally increases root longevity (Ryser, 1996; Eissenstat *et al*., 2000; Eissenstat and Volder, 2005), which may support root survival through the dry season due to an associated decrease in root respiration (Eissenstat *et al*., 2000; Eissenstat and Volder, 2005). Recent research has also demonstrated that in African savannas there is a flush of nitrogen that becomes available with the first rains at the end of the dry season (February and Higgins, 2016). With a well-established fine root system *Senegalia* is able to respond rapidly and aggressively to the increased availability of nutrients, such as nitrogen at the beginning of the wet season. In addition, long-lived roots would enable *Senegalia* to better exploit soil resources associated with grass-root die-back through the dry season (McConnaughay and Bazzaz, 1992). The production of long-lived roots does, however, increase maintenance costs that may account for the slower growth rates of some *Senegalia* (Eissenstat and Volder, 2005).

This trade-off would suggest that where *Senegalia* seedlings are able to establish as juveniles and release into adult size classes when grass biomass is relatively high, *Vachellia* juveniles can only establish and release as adults when grass biomass is reduced. The coexistence of *Vachellia* and *Senegalia* in savannas may, therefore, be related to differences between the two genera in

establishing into adult size classes in association with competition from grasses for resources. Following successful seedling establishment in the same safe-sites among grasses *Vachellia* has the risky strategy of putting all its resources into rapid growth while *Senegalia* is slower growing with increased root tissue density (Fig. 4). In this scenario, because *Vachellia* release relatively faster as adults when grass biomass is reduced and because disturbance of the grass layer is a major initiate of tree invasion into savannas (Bush and Van Auken, 1995; Davis *et al*., 1998, 2000; Ward and Esler, 2011; O'Connor *et al*., 2014) this scenario could thus, explain why *Vachellia* is observed to be relatively more invasive than *Senegalia*.

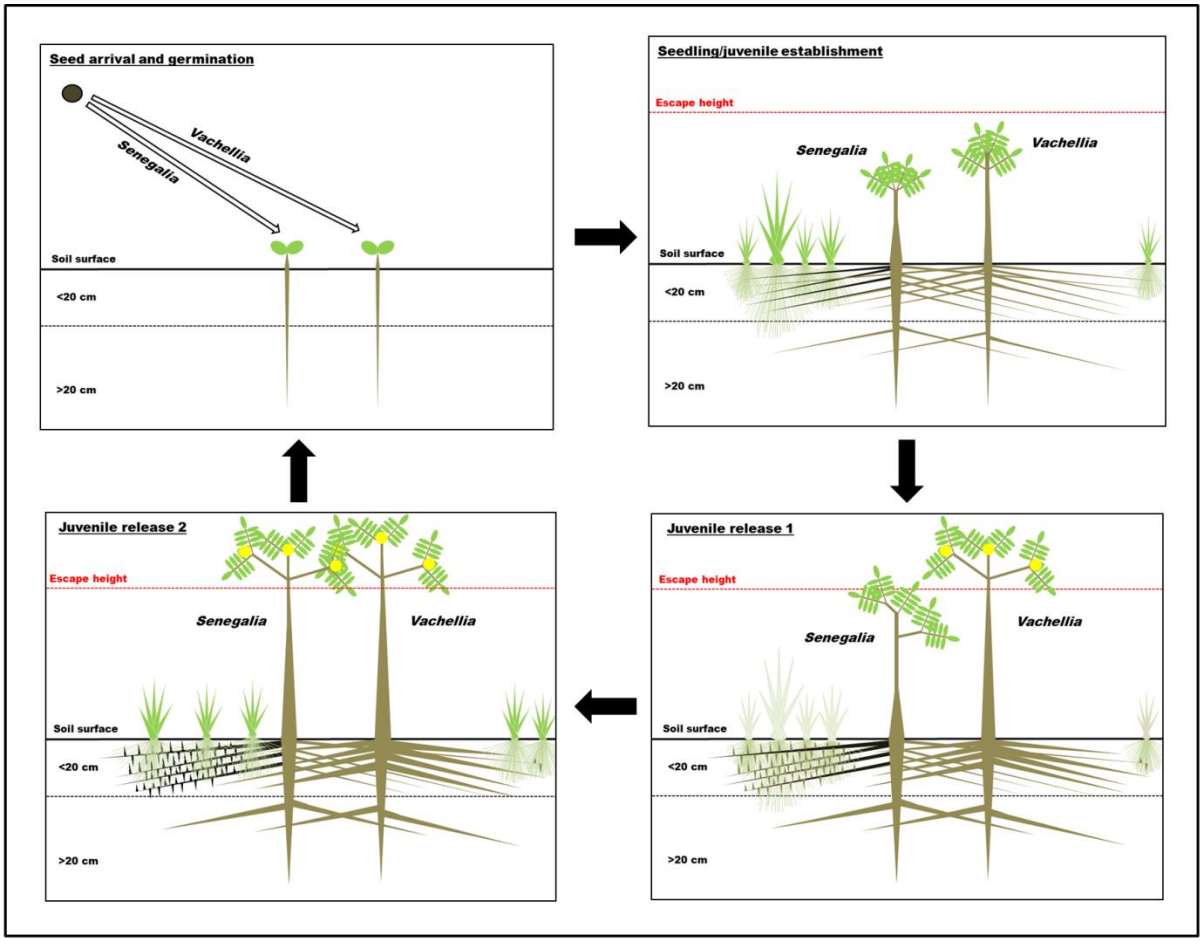

Figure 4: Illustrating the hypothesised mechanism of Eltonian niche partitioning between *Vachellia* and *Senegalia* species in a variable grass layer. Highlighting that *Vachellia* juveniles rapidly release from increased investment into vertical height when grass biomass is reduced but, the slow growing *Senegalia* juveniles steadily release because they compete directly with grasses for edaphic resources. Increases in root tissue density (≈ longevity) in roots is annotated in black. Dead grasses are shown as pale grey.

I expected competition with grasses to result in seedlings from both genera investing more heavily in root biomass and more conservative tissues (i.e. shorter roots per unit weight and heavier leaves per unit area). I expected this because belowground competition from grasses should result in declining water and nutrient availability, which usually results in higher root-to-shoot ratios and smaller

heavier leaves (Ryser, 1996; Cornelissen *et al.*, 2003; Díaz *et al.*, 2004; Eissenstat and Volder, 2005b; Poorter *et al.*, 2009; de la Riva *et al.*, 2016). Contrary to this expectation, however, I found no increase in root-to-shoot ratio and investment into longer roots per unit weight and lighter leaves per unit area in the seedlings of both genera when growing among grasses. These results agree with studies that have demonstrated that competition with grasses may lead to reductions in available water and nitrogen resulting in reduced leaf mass ratios (Loomis, 1997; Provendier and Balandier, 2008). These results are also in agreement with studies that have demonstrated that thinner and more ramified roots in the presence of grasses correspond with a foraging strategy to exploit soil resources (Bauhus and Messier, 1999; Curt *et al.*, 2005).

These results would suggest that both genera are outcompeted for resources by grasses and have adapted physiologically to compensate for this (Brisson and Reynolds, 1997). While both genera likely compete for similar safe-sites when establishing, *Vachellia* avoids competing with grasses for resources by establishing into gaps in the grass root-mat and before grass biomass can recover, rapidly growing in height to adult size classes. *Senegalia*, by contrast, is slower growing and competes directly with grasses for resources by increasing root survival among the grass root-mat but, suffers slower vertical growth as a consequence. This mechanism of Eltonian niche partitioning suggests that, where the release of *Vachellia* juveniles into adult size-classes is more tied to times of low grass biomass, the release of *Senegalia* juveniles is steadier over time. This mechanism is, supported by Staver *et al.* (2011) who demonstrated that release of *V. karroo* and *V. nilotica* juveniles into adult size-classes was far more pulsed than for *S. nigrescens*, which was not significantly tied to any particular time (i.e. steady). Future research should determine if the divergent seedling strategies identified in this study do lead to differences in juvenile release in the field between these genera. This mechanism suggests that controlling invasive *Vachellia* requires different management practices to those necessary for controlling invasive *Senegalia.* Where increasing grass biomass to preclude juvenile release might best control invasion by *Vachellia*, controlling the invasion of *Senegalia* may be better served by using the more traditional means of fire and herbivory.

Supplementary Material

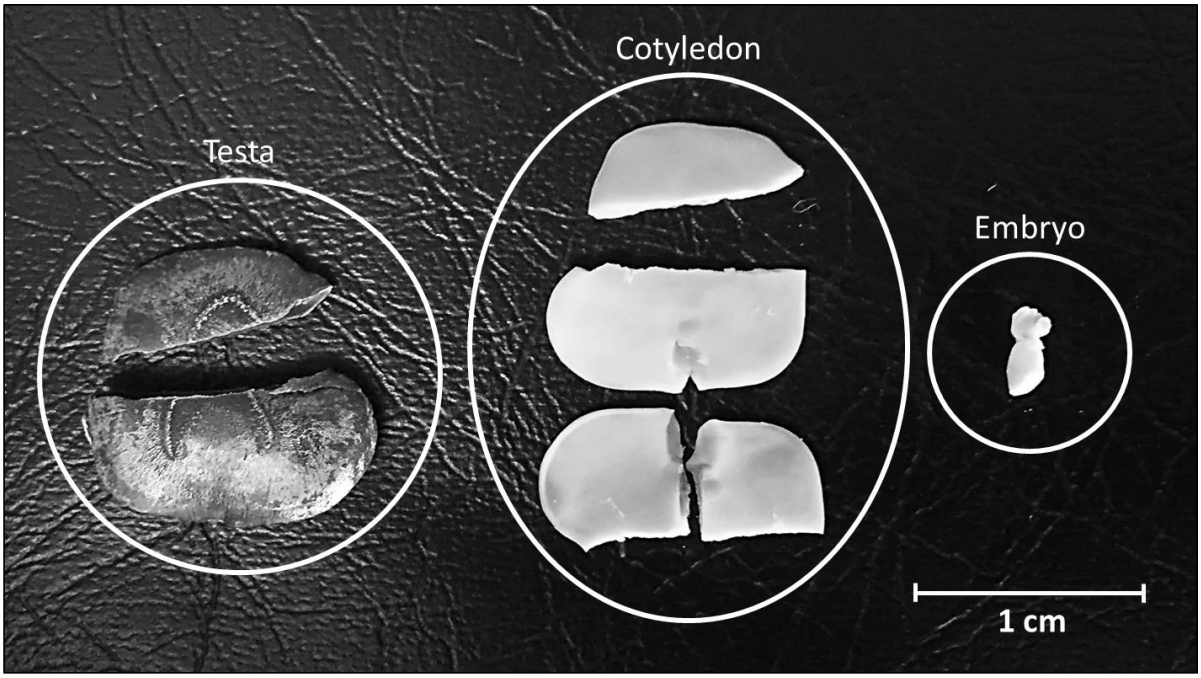

Figure 1: Photograph of a dissected *Senegalia montis-usti* seed. Illustrating the different components of seeds which were separated.

Table 1: Highlighting the change in watering regime throughout the duration of the glasshouse experiment to simulate dry conditions in the field during winter.

	Regime	Duration					Additional Handwatering
		5 min	10 min	15 min	20 min	25 min	
Day 0-13	1 times per day		✓				
Day 14-84	1 times every 2nd day		✓				
Day 85-176	1 times per week					✓	
Day 177-183	1 times per week			✓			
Day 184-224	1 times per week	✓					
Day 225-231	2 times per week	✓					
Day 232-251	2 times per week			✓			
Day 252-END	2 times per week				✓		✓

Chapter 4:

Synthesis

In this thesis, I tested the hypothesis that *Vachellia* and *Senegalia* have undergone ecological divergence in savannas since their evolutionary separation 30 Ma, and that this underpins both niche partitioning and observed differences in invasiveness between these genera. In support of this, while I find limited Grinnellian niche differentiation between these genera at both a regional and landscape scale, a general pattern of coexistence at the plot-scale highlights their Eltonian niche partitioning (see also Fig. 1 & Fig. 2). After conducting a large glasshouse experiment, I find it unlikely that their plot-scale coexistence is enabled by water-source partitioning or by differential survivorship among grasses during seedling establishment. Observation of similar stresses to seedlings establishing among grasses, coupled with an improbability that dispersal differences between *Vachellia* and *Senegalia* significantly affects their seedling establishment, means that these genera likely compete for the same safe sites. Demonstration that *Vachellia* seedlings grew significantly taller when growing without the presences of grasses provides a functional explanation for why this genus is observed to be more vigorously invasive (O'Connor *et al.*, 2014). Greater vertical growth rates in *Vachellia* free from grass competition increases invasiveness because disturbance, which reduces grass biomass, is a primary determinant of invasion (Bush and Van Auken, 1995; Davis *et al*., 1998, 2000; Ward and Esler, 2011; O'Connor *et al*., 2014) and greater height growth will mean juvenile *Vachellia* reach adult size classes relatively faster in these situations (Trollope, 1984; Bond and van Wilgen, 1996; Higgins *et al*., 2007; Sankaran *et al*., 2013; Staver and Bond, 2014). *Senegalia* seedlings increase root tissue density in response to grass-root competition, which results in increased root longevity during the dry season (Eissenstat *et al*., 2000; Eissenstat and Volder, 2005). This strategy provides *Senegalia* with an opportunity to respond more aggressively to the pulse of nutrients that becomes available at the start of the next growing season (February and Higgins, 2016). Eltonian niche divergence between these two genera in savannas is thus enabled by grasses, which prevent the rapid release of juvenile *Vachellia* and provide juvenile *Senegalia* with a chance to better exploit grass-root turnover. The phylogenetic community structure of *Vachellia* and *Senegalia* and their functional differences as seedlings, therefore, overwhelmingly support the hypothesis that these two genera are divergent in ecological niche and furthermore, that this divergence underpins differences in invasiveness.

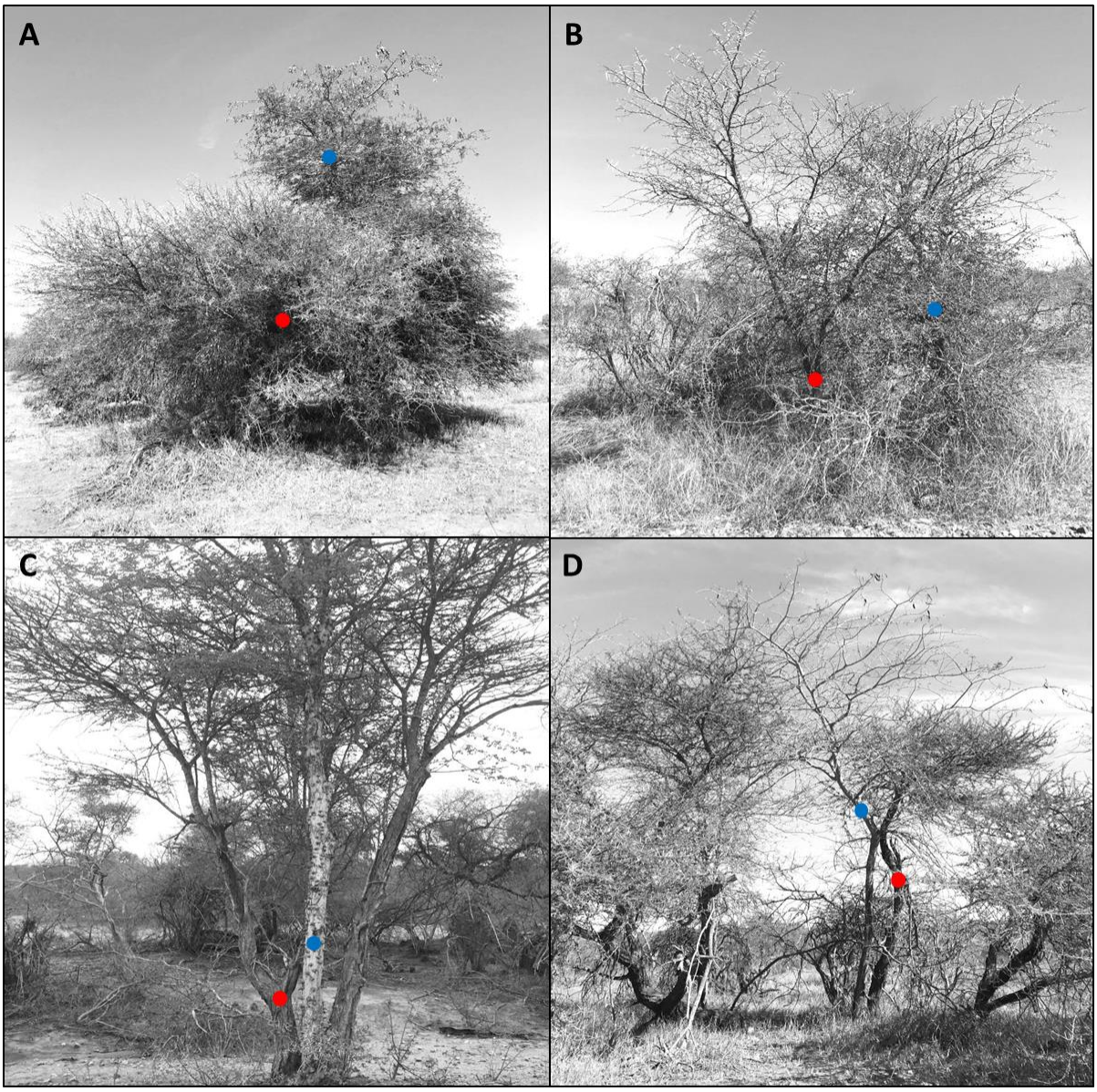

Figure 1: Photographs showing safe-site sharing at the plot-scale between adult *Vachellia* (red) and *Senegalia* (blue) species. Mature *V. tortilis* and *S. welwitschii* (**A** and **D**), *V. exuvialis* and *S. nigrescens* (**B**) and *V. tortilis and S. nigrescens* (**C**). Photographs were all taken between the Olifants River and Skukuza in the Kruger National Park, South Africa.

The mechanism of Eltonian niche partitioning proposed here for *Vachellia* and *Senegalia* (Fig. 4, Chapter 3) is tentatively supported by Staver *et al*. (2011). These authors show that juvenile release in some species of *Vachellia* is episodic, while that of *S. nigrescens* is steady through time. Where these authors argue that fire and herbivory are responsible for differences in the juvenile release behaviour of these species, they did not consider the effect of grass competition. Grass competition, however, increases with the succession of grass species, pioneer-to-climax, after a disturbance event (van Oudtshoorn, 2012; Lewis and February, unpublished). The strongly episodic nature of juvenile

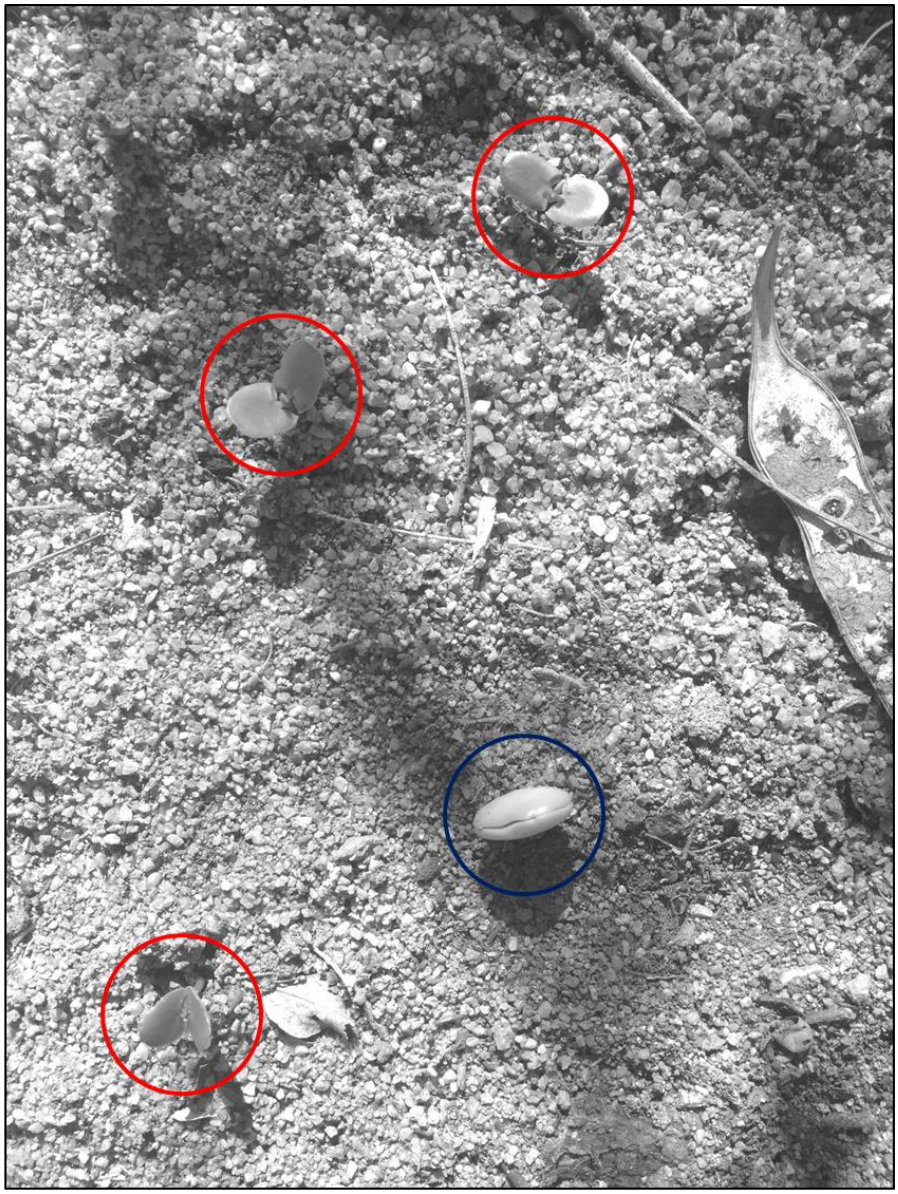

Figure 2: Photograph showing spatiotemporally synchronous germination between *Vachellia tortilis* (red) and *Senegalia nigrescens* (blue). Photograph was taken two days after the 2016 March rains in the Skukuza Rest Camp, Kruger National Park, South Africa.

release in *V. karroo* and *V. nilotica* noted by Staver *et al.* (2011) may, therefore, reflect the association of this genus with establishment opportunities arising when the grass layer is poorly developed following significant disturbance. This may explain why species of *Vachellia* overwhelmingly dominate the pastoral landscape (overgrazed by cattle) bordering the eastern edge of Hluhluwe-iMfolozi Park, South Africa, despite the presence of large stands of reproductive *S. nigrescens* and *S. burkei* trees (pers. obs.). Some species of *Vachellia*, such as *V. xanthophloea* (Young and Lindsay, 1988), *V. nilotica* (Bond *et al.,* 2001), *V. gerrardii* (pers. obs. Nkuhlu Exclosure,

KNP) and *V. robusta* (pers. obs. Olifants River, Balule Private Nature Reserve), also exhibit conspicuous cohorted senescence in the field, which may be related to the pulsed release of juveniles into adult size-classes (Young and Lindsay, 1988). That *Vachellia* and *Senegalia* employ different recruitment strategies may explain why recruitment in African *Acacia s.l.* has previously been inconsistently characterized as steady or cohorted (Midgley and Bond, 2001).

To explain niche partitioning between *Vachellia* and *Senegalia*, these divergent strategies would have to be conserved through evolutionary time (Losos, 2008). The evolution of *Vachellia* and *Senegalia* would, therefore, have to be such that *Senegalia* is less likely of rapid vertical growth and *Vachellia* is less likely to have an adaptive root response. In support of this, it has long been recognised that *Vachellia* and *Senegalia* show evolutionarily-conserved differences in physical defences (Robbertse, 1975a; Ross, 1979). Where *Vachellia* species all possess formidable spines derived from stipules, while species of *Senegalia* possess robust prickles derived from swellings of the stem epidermis (Robbertse, 1975a; Ross, 1979). The major consequence of this difference is that where the spines of *Vachellia* are directly connected to the vascular system, the prickles of *Senegalia* are not (Robbertse, 1975a). Connection to the vascular system may therefore underpin the responsiveness of the defences of *Vachellia* to both resources (Gowda *et al.*, 2003) and herbivory (Gowda, 1997; Young *et al*., 2003), rather than age, as with the epidermal prickles of *Senegalia*. This difference is important because the more responsive (aggressive) physical defence in *Vachellia* may have been a crucial exaptation for the evolution of dwarf lateral shoots in this genus (Robbertse, 1975b; Ross, 1979), given that physical defence in savanna trees is more likely concerned with the protection of stem nodes rather than leaves (Midgley *et al*., 2001). Dwarf lateral shoots are very small non-woody shoots (fascicles) arising at the nodes which bear secondary (proximal) leaves and preclude the production of additional woody tissue to increase leaf area. This condition is highly developed in *Vachellia* (see Fig. 3) and likely allows these species to invest considerably in vertical, rather than lateral, growth. By contrast, the prickles, of *Senegalia* are modified climbing structures which reflect a long history of scandent growth in this genus (*Aculeiferum*; Ross, 1981), which requires the continual growth of lateral branches, perhaps reducing the usefulness of dwarf lateral shoots in these species (Ross, 1979). Climbing species also require adequate substrate (vegetation) to gain purchase on and climbers using prickles are especially reliant on an established mass of tangled vegetation (Darwin, 1867). This necessity for climbing over dense vegetation inevitably incurs a cost in terms of below-ground competition and may thus explain why *Senegalia* was seen to have an adaptive root response to grass-root competition when *Vachellia* was not (Hodge, 2004).

Figure 3: Photograph of *Vachellia robusta* illustrating the highly-developed condition of heterophylly in this genus. The axillary buds, giving rise to dwarf lateral shoots, are so numerous at the nodes that they appear like cushions.

Evidence for Eltonian niche partitioning between *Vachellia* and *Senegalia* suggests that intra-generic resource competition could be strong enough to shape plot-scale communities. This has already been suggested for Floridian oak savannas, where different oak lineages avoid resource competition coexisting at the plot scale by germinating in different years (Cavender-Bares *et al.*, 2004). Many researchers, however, argue that tree community structure in African savannas is primarily driven by demographic bottlenecks (habitat filters), such that tree-tree competition is assumed to be negligible (Higgins *et al*., 2000; Bond *et al*., 2001; Midgley and Bond, 2001; Bond, 2008; Staver *et al*., 2012; Charles-Dominique *et al*., 2015a, 2015b). Despite this argument, several studies have suggested that tree-tree competition could determine species assembly in plot-scale communities in African savannas. For example decreasing nearest-neighbour distances (Smith and Walker, 1983; Smith and Goodman, 1986; Shackleton, 2002; Belay and Moe, 2012) and increased neighbourhood density (Wiegand, Ward and Saltz, 2005; Sea and Hanan, 2012; Dohn *et al.*, 2017) significantly reduce tree growth or, lead to competitive exclusion (Colgan and Asner, 2014). Other studies show

that nearest neighbour removal greatly increases tree growth (Smith and Goodman, 1986; Kambatuku *et al*., 2010), that increased tree size leads to regular spatial patterning through self-thinning (Smith and Walker, 1983; Meyer *et al.*, 2007) and also that high adult tree densities negatively influence seedling establishment (Smith and Goodman, 1986; Smit, 2004; Pillay and Ward, 2014). Decreasing nearest neighbour distances have also been observed to significantly increase tree mortality (Moustakas *et al.*, 2008). It is imperative for future researchers to determine if tree-tree competition is structuring communities, as only the balanced competition model of savanna structure currently argues that tree-tree competition is significant (Scholes and Archer, 1997).

Future research is needed to determine whether the divergent seedling strategies identified in this study do indeed underpin differences in juvenile release into adult size-classes explaining differences in the relative invasiveness of *Vachellia* and *Senegalia*. This is important to establish as a difference in juvenile release would imply that the threat posed by species of *Vachellia* needs to be managed differently to that posed by *Senegalia*. The bolting strategy of Vachellia may leave the traditional management of bush encroachment (fire and/or herbivory) inadequate because, if already established as juveniles, the rapid growth of *Vachellia* would allow these species to establish into adult size classes. To prevent this management should consider promoting high grass biomass, which might preclude seedling/juvenile establishment. Controlling invasive *Senegalia*, however, may be best served by continuing traditional practices as, increased grass biomass is unlikely to stop these species establishing as juveniles but, because of their slow growth, traditional practices may stall release indefinitely. This is a critical research area in the present time as, bush encroachment is currently leading to enormous losses of biodiversity in savannas worldwide (Ward, 2005; Ratajczak *et al*., 2012; O'Connor *et al*., 2014; Stevens *et al*., 2017).

Field based verification may be accomplished through spatial point pattern analysis of mature adult and senescent adult trees along transects. If *Vachellia* species are exhibiting more pulsed juvenile release than *Senegalia* species, the expectation is that *Vachellia* adults and senescent adults should be spatially and temporally more clumped than those of Senegalia. In addition, the approach of Staver *et al.* (2011) should be used to assay a broader suit of species from both genera, but especially *Senegalia* which is only represented by a single species in their study. Further research is also needed to identify the causes and consequences of the root tissue density response observed here in *Senegalia*. While short-term split-pot experiments may provide a good test of root responses to grass-root competition, longer-term (i.e. > 1 yr) glasshouse experiments will be needed to determine whether this response significantly influences biomass accumulation (Ryser, 1996). In

these experiments it will also be important to determine the density-response of fine roots specifically, as these are the roots actively involved in nutrient uptake and root proliferation (Pregitzer, 2002).

Demonstration of plot-scale coexistence between *Vachellia* and *Senegalia* species around around 24° 25'S latitude suggests that coexistence between these genera should be actively monitored in KNP to ensure ecosystem stability and resilience (McCann, 2000; Hooper *et al.*, 2005). Managing this coexistence if it degrades may be done by first identifying which species most commonly coexist in particular habitats (e.g. *S. nigrescens* and *V. tortilis* in the stunted knob-thorn savanna) and then sowing mixed seeds or planting out juveniles, from both genera, into the same safe sites and subsequently maintaining a variable grass layer. Further research is, however, needed to assess whether the coexistence of these genera in plots is a general trend for *Vachellia-Senegalia* plot-scale communities in African savannas or if it is specifically restricted to semi-arid savannas and/or this national park. Similar methods to those used in Chapter 2 of this study would be sufficient to determine coexistence; however, care must be taken to keep plot sizes small enough to capture ecological interactions between individuals.

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